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1941

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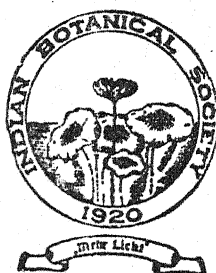
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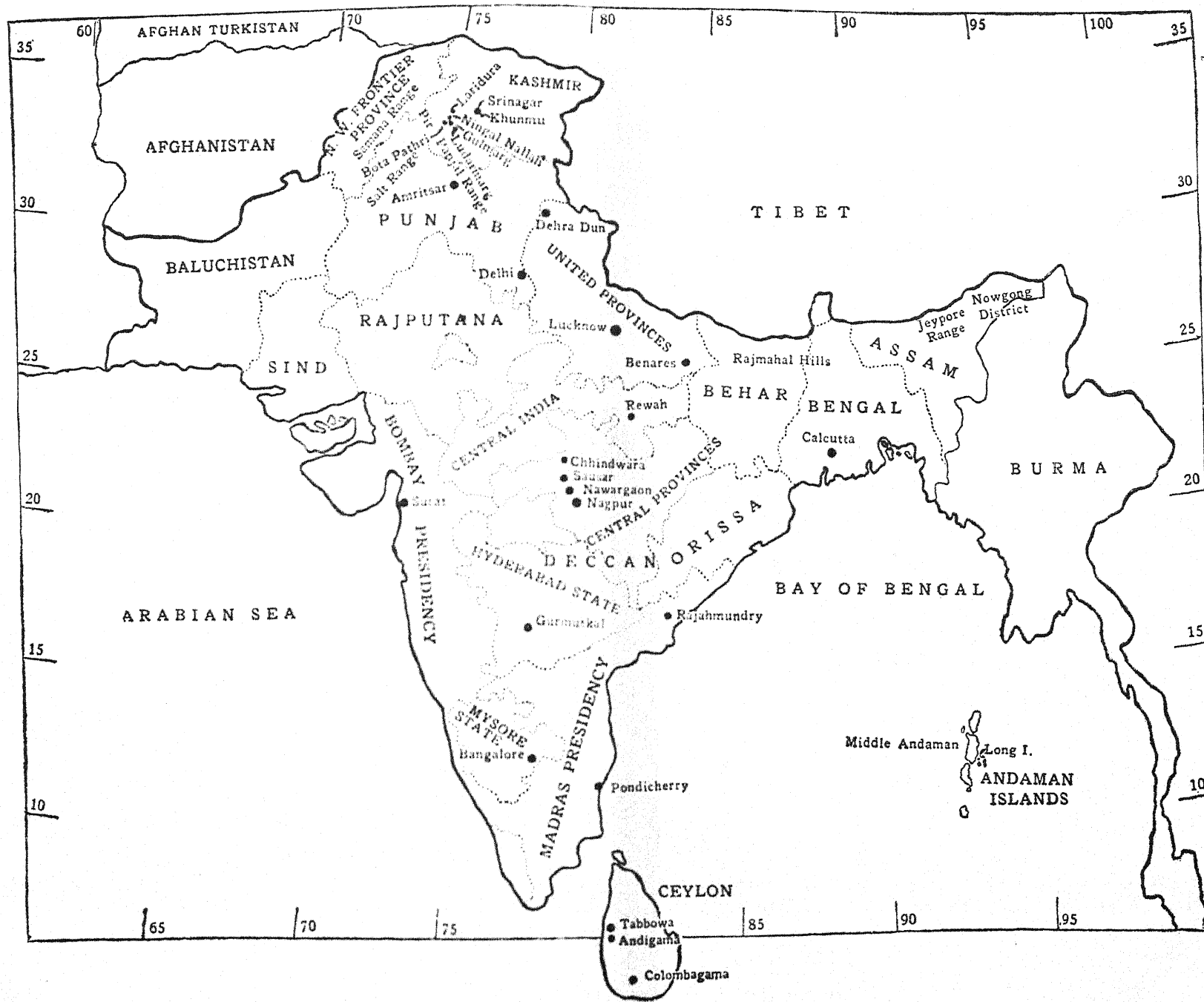
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II

Progress Report for 1940

NOTE

THESE reports are published with the co-operation and authority of a working committee. They are based upon data for which the members are severally responsible, the convener acting as an editor.

Colleagues desiring to receive the reports should send a request to the convener, who will be glad to place their names on the mailing list.

Department of Botany,
The University,
Lucknow, U.P., India,
January 1941.

B. SAHNI,
Convener.

CARBONIFEROUS

Salt Range.—Mrs. Jacob of Calcutta (formerly Miss C. Virkki, Lucknow) has completed her work on the flora of the early Gondwana beds in the Salt Range. The memoir has been sent to the press under the following title :

Mrs. C. Jacob (C. Virkki), "A Lower Gondwana flora from the Salt Range, Punjab". (MS. 82 pp., 17 Pls., 11 Text-figs.). Publication expected in *Palæontologia Indica* (Geological Survey of India, Calcutta).

For a brief notice of the contents see the Report for 1939 (*Journ. Ind. Bot. Soc.*, 18, 202).

CARBONIFEROUS AND PERMO-CARBONIFEROUS

India and Australia.—Mrs. Jacob's work on the microflora of certain Lower Gondwana strata in India and Australia has likewise been sent to the press :

Mrs. C. Jacob (C. Virkki), "Spores from the Lower Gondwanas of India and Australia". (MS. 108 pp., 14 Pls., 79 Text-figs.). Publication expected in *Palaeontologia Indica* (Geological Survey of India, Calcutta).

This is a rather comprehensive work describing mostly spores, but also a few other plant-remains as well as a few arthropod chitins, from various Lower Gondwana localities in India and Australia. The spores are of many different shapes and sizes, and some are winged, others unwinged. The wings vary in their form and number; one circular wing all round, or two wings as in the genus *Pityosporites*, or three wings symmetrically placed. An interesting fact is that a continuous series of transitions can be traced between the two-winged type of spore and the type with one circular wing passing all round the spore (see G. Dubois, *Chronica Botanica* V, 4/6, 1939, p. 408 and references therein). An artificial key to the identification of the many kinds of spores is given. An important fact recorded in the Report for 1939, was the discovery, in the Bacchus March tillite, of several kinds of spores indistinguishable from those found in such far scattered localities in India as the Salt Range, the Dalongunj coalfield in Bihar, and the Rewah basin in Central India. The significance of these occurrences is now discussed in connexion with the climatic relations of the early *Glossopteris* flora and the possible use of these spores in Gondwana stratigraphy.

PREMO-CARBONIFEROUS

Dalongunj Coal-field (Behar).—Mrs. Jacob has sent to press the following paper on the cuticle of *Glossopteris communis* Fst.:

Mrs. C. Jacob (C. Virkki), "The cuticular structure of *Glossopteris communis* Feistmantel". (MS. 11 pp., 2 Pls.). Publication expected in *Palaeontologia Indica* (Geological Survey of India, Calcutta).

G. communis Fst. was united with *G. indica* Schimp. by Zeiller in 1896, but the cuticular structure of *G. communis* as observed by Mrs. Jacob is distinct from that of *G. indica* as described by Zeiller. This suggests that it is better to keep the two forms separate. Adhering to the under side of the lamina masses of two-winged spores of the *Pityosporites* type were also found. These are described in a separate paper already noticed above.

Kashmir.—From Lower Gondwana beds at Dandlutar, in the Pir Panjal Range, R. V. Sitholey (Lucknow) has described several fragments of the leaves of *Psygmoptyllum Haydeni* Seward which have enabled him to attempt a reconstruction of the leaf-bearing shoot in this species. This indicates a close general similarity in habit to *Ginkgo biloba*, although there is no evidence of short shoots. A paper has been sent to press with the following title:

"*Psygmoptyllum Haydeni* Seward from a new locality in Kashmir". (MS. 8 pp., 2 Pls., 3 Text-figs.). Publication expected in the *Records of the Geological Survey of India*, Calcutta.

B. Sahni with Messrs. S. L. Raina, B. L. Kaw and A. N. Fotidar of Srinagar made a collection of Lower Gondwana plants from the *Gangamopteris* beds near Khunmu in the Vihi Valley, a few miles S. E. of Srinagar. The specimens were lodged in the Biology Department of the Sri Pratap College, Srinagar.

PERMIAN

Brazil.—H. S. Rao's work on *Lycopodiopsis Derbyi* has been published :

"On the anatomy of *Lycopodiopsis Derbyi* Renault, with remarks on the southern Palæozoic lycopods." *Proc. Ind. Acad. Sci.*, Vol. XI, No. 5, Sec. B, pp. 197-217.

TRIASSIC

Salt Range.—The Triassic flora from this area which R. V. Sitholey (Lucknow) is investigating, has turned out to be much richer in species than was previously suspected from the extremely fragmentary nature of the plant-remains. Among the impressions of fern pinnae and bits of stems, reported last year, the following genera had already been recognised :—*Equisetites*, *Sphenopteris* (several species) and *Cladophlebis*. One specimen of a *Sphenopteroid* pinna (in two counterparts) bears on the under side of several of its pinnules epaulette-shaped sori. From the vicinity of one of the pinnules a mass of spores has been obtained by maceration. Bulk maceration of the rock has yielded casts of several types of large spores bearing a very prominent triradiate mark. Some of these are being described as new species of *Triletes*. Other spores or spore-like bodies of smaller size are being provisionally referred to *Sporites*. Many of the spores, large and small, are partially enclosed in a thick cup-like envelope which closely invests each spore, usually leaving a wide opening from which the triradiate mark emerges. In addition a few cuticles have been prepared.

JURASSIC

Afghan-Turkistan.—R. V. Sitholey's memoir on C. S. Fox's collection of plants from Afghan-Turkistan has been published :

"Jurassic plants from Afghan-Turkistan." *Palæontologia Indica*, Vol. XXIX, Memoir No. 1, 25 pp. (with 8 Pls., 7 Text-figs. and one Map).

Rajmahal Hills.—K. Jacob (Calcutta) has sent to the press the following works on the Jurassic flora of this region :

"Fossil plants from Sakrigalighat in the Rajmahal Hills, with remarks on the age of the beds." (MS. 61 pp., 11 Pls., 52 Text-figs.). Accepted for publication in *Palæontologia Indica* (Geological Survey of India, Calcutta).

"On the structure and affinities of *Tinpaharia*, a new genus of petrified ferns from the Rajmahal Hills." (MS. 57 pp., 27 Pls.,

19 Text-figs.). Accepted for publication in *Palaeontologia Indica* (Geological Survey of India, Calcutta).

Both these papers have been briefly noticed in the Report for 1939, p. 204.

A. R. Rao (Lucknow) has sent to press the following papers, also briefly noticed in the Report for 1939 :

"The structure and affinities of *Teniopteris spatulata* McClelland." (MS. 35 pp., 10 Pls.). Accepted for publication in *Palaeontologia Indica* (Geological Survey of India, Calcutta).

"Two petrified strobili from the Rajmahal Hills, Behar." (MS. 25 pp., 5 Pls.). Accepted for publication in *Palaeontologia Indica* (Geological Survey of India, Calcutta).

He is at present engaged in describing some petrified shoots, and some winged spores which occur scattered in the matrix of the blocks.

K. M. Gupta (Surat) has described a well preserved specimen of *Williamsonia* from Khairbani, near Mirazchowki Railway station, Behar. The mode of preservation is interesting, because parts of the specimen are seen as impressions (*e.g.*, the spreading ovate lanceolate bracts on which the ramental scales are clearly shown), while the seminiferous and interseminal scales are preserved in the solid form as a petrification though the details of their anatomy are not seen. The distal ends of the scales have left their impressions on the rock as an unusually well preserved mosaic pattern closely comparable with that described in *W. Sewardiana* Sahni, with which the specimen shows several other points of close resemblance. Thus, the form and size of the receptacle, the length of the seminiferous and interseminal scales, and the form of the rametal scales, are also very similar to the corresponding organs in that Rajmahal species, which is itself closely allied to *W. scotica* Seward. Other species with which comparisons are possible are the Mexican forms *W. xicotencatl* Wiel., *W. cauhtemoc* Wiel. and *W. Nathorsti* Wiel. The flower is either specifically identical with *W. Sewardiana* or very closely allied to it.

Ceylon (Tabbowa).—K. Jacob (Calcutta) has sent the following paper to the press :

"Jurassic plants from Tabbowa, N.W. Ceylon." (MS. 23 pp., 7 Pls., 4 Text-figs.). Publication expected in *Palaeontologia Indica* (Geological Survey of India, Calcutta).

See Report for 1939, p. 205.

A further collection of fossil plants from the same beds, kindly sent by D. N. Wadia of the Mineralogical Department, Colombo, has been received for investigation at Lucknow.

Ceylon (Andigama).—Some fragments of carbonaceous shale from Andigama in the Tabbowa district were received from D. N. Wadia, and others from P.E.P. Deraniyagala, Curator of the Colombo Museum, to both of whom we owe other contributions of material

reported here. Miss M. Janet (Lucknow) has recovered by maceration from these shales a considerable variety of microscopic remains of plants as well as animals. The microflora includes several kinds of spores, winged and unwinged; shreds of coniferous wood with bordered pits; some cuticles and a few filamentous plants of unknown affinity, possibly fungus mycelia. Among the animal remains are fragments of the chitinous exoskeletons of arthropods, chiefly appendages. The work is still at a very early stage and none of the forms can yet be named, but it is worthy of note as the first contribution to our knowledge of the fossil microflora (and microfauna) of Ceylon.

CRETACEOUS ? AND TERTIARY ?

Assam.—The investigation of the algal flora from some limestones of doubtful Cretaceous or Eocene age sent by the Geological Survey of India for study, has now been completed by K. S. Rao (Bangalore). The detailed report, which is awaiting publication, contains descriptions of two new species of *Archæolithothamnium*.

CRETACEOUS ? OR TERTIARY ?

Hyderabad (Deccan).—K. S. Rao (Bangalore) has completed the study of the fossil Charophyta of the Intertrappean beds recently discovered near Gurmutkal, Gulbarga District, Hyderabad State. It is of interest to record that the species now being described are different from those previously reported from other Intertrappean beds of India.

TERTIARY

Deccan (Intertrappean Beds: General).—Palæobotanical evidence for an early Tertiary age of these beds, and therefore of the Deccan Traps, is steadily increasing. It will be recalled that the early geologists Malcolmson, Hislop and Hunter had placed these beds in the Eocene, mainly on the fossil evidence. This view was later abandoned, and until recently the Deccan Traps were commonly regarded by geologists as more probably Upper Cretaceous. A Tertiary age was, however, indicated by Smith Woodward's work (1908) on some fish remains from the Deccan and more recently by the work of S. L. Hora (1938). Towards the end of 1933 the Tertiary theory was definitely revived by a preliminary review of the fossil flora of these beds in the Central Provinces. Several of the works noticed below go in support of this idea.

In a presidential address entitled "The Deccan Traps: an episode of the Tertiary Era" (*Proc. 27th Indian Science Congress, Madras, Jan. 2, 1940, pp. 1-21, with 2 Pls. and one map*), B. Sahni attempts to visualise the physical conditions during the volcanic era in the Deccan and then proceeds to give a cursory account of the organic remains. He concludes that "From what we know of the geological history of the stoneworts, the fungi, the water-ferns and particularly of the palms, which formed such a vast

proportion of the flora, everything seems to point to a Tertiary age. What is more, the fishes and the crustaceans, too, seem to fall into line with the plants."

Sausar, Chhindwara District (Intertrappean Beds).—The paper by B. Sahnii and H. S. Rao, on the flora of some Intertrappean cherts from Sausar, has been sent to the press under the following title :

"The silicified flora of the Deccan Intertrappean Series : the flora of the Intertrappean cherts in the Sausar Tehsil, Chhindwara District, Central Provinces". (MS. 88 pp., 7 Pls., 19 Text-figs.). Publication expected in *Palæontologia Indica* (Geological Survey of India, Calcutta).

Central Provinces (Intertrappean Beds).—V. B. Shukla (Nagpur) has described from the Chhindwara District a well preserved dicotyledonous wood with a structure closely comparable with that of the modern genus *Casuarina*. A new species of *Palmoxylon* (*P. nawargaoensis*) is named after Nawargaon in the Wardha District. Further material of fossil wood from the Intertrappean beds of the Chhindwara District is under investigation.

Rajahmundry (Intertrappean Beds).—The memoir on the fossil Charophyta of Rajahmundry mentioned in the Report for 1939, p. 206, has now been published :

K. S. Rao and S. R. N. Rao (Bangalore), "The fossil Charophyta of the Deccan Intertrappeans near Rajahmundry (India)", *Palæontologia Indica* N.S. Vol. XXIX, Mem. 2, pp. 1-14, Pls. I-III, Calcutta.

The authors describe and figure 13 species under the genus *Chara* of which the following four are regarded as new : *C. rajahmundryica*, *C. Sampathi*, *C. Sahnii* and *C. indica*. It is significant that most of the other species are identical with those from the Lower Headon beds of Hurdle Cliffs, Hampshire, described by Reid and Groves, and considered to be of Eocene age.

In a paper read before the 27th Indian Science Congress at Madras in January 1940 a new species of Dasycladaceæ was described under the name *Dissocladella intertrappea*. Published in abstract :

S. R. N. Rao and K. S. Rao, "More algæ from the Rajahmundry Intertrappeans". *Proc. 27th Ind. Sci. Congress (Madras Session, Section of Geology)*. Pt. III (Abstracts), pp. 118-19, Calcutta.

North-West Frontier Province.—The following paper has been published in abstract form :

S. R. N. Rao (Bangalore), "An algal flora from the Lockhart Limestone (Ranikot series) of the Samana Range (N.W. Frontier Province)". *Proc. 27th Ind. Sci. Congress (Madras Session, Section of Geology)*. Pt. III (Abstracts), p. 119, Calcutta.

Three new species have been described and figured in this paper: *Lithophyllum lockharti*, *Archæolithothamnium ranikotensis* and *A. samanensis*.

Sind.—S. R. N. Rao (Bangalore) is preparing a detailed account of a species of *Lithophyllum* which he discovered in a thin section of a foraminiferal limestone of Upper Ranikot age, from Jhirak, Sind. The material comes from the Geological Survey collections. The present species is different from *L. Lockharti* Rao, previously described from the Ranikot beds of the Samana Range (N. W. Frontier Province). A Dasycladacea, *Bröckella ranikotensis* Walton, previously described under the name *Triploporella ranikotensis* Walton (1926), is already known from the Ranikot beds of Sind.

Surat.—S. R. N. Rao (Bangalore) has discovered fragments of *Lithothamnium* in thin sections of the *Pellatispira* limestone from the Nummulitic series near Surat. From a study of the foraminifera, he considers the limestone to be of Upper Eocene (Auversian) age and he is at present engaged in an investigation of the algal contents.

Andaman Islands.—In 1926 E. R. Gee described and figured a species of *Archæolithothamnium* (not *Lithothamnium nummuliticum*) from the *Lepidocyclus* limestones of Miocene age from Long Island, near Middle Andaman. In sections of the same limestone, which S. R. N. Rao (Bangalore) obtained from the Geological Survey of India, he has recognised several species of algæ of the genus *Amphiroa*, which has not so far been recorded from India. *Amphiroa* is of frequent occurrence in post-Eocene limestones in other parts of the world. A report on the algæ studied is under preparation.

Behar.—K. A. Chowdhury (Dehra Dun) has received for detailed study 27 specimens of petrified wood of Tertiary origin, collected by A. K. Banerjee of the Geological Survey of India.

Assam.—K. A. Chowdhury and his co-workers at Dehra Dun are studying a large collection of fossil dicotyledonous woods from various Tertiary localities. The collection includes one specimen from the Jeypore Range in the Lakhimpur district, 20 specimens collected by P. R. Datta of the Assam Forest Service in the Nowgong district, 31 specimens collected by K. B. Mohanlal of the Indian Forest Service in the bed of the Tailangthu Nadi (river) and 47 specimens collected by K. A. Chowdhury at Diphu, on the Assam-Bengal Railway.

Burma.—Six specimens of fossil dicotyledonous wood, collected from the Pakokku Meiktila and Shwebo districts of Burma and sent by M. N. Gallant of the Indian Forest Service, are also being investigated by K. A. Chowdhury.

PLEISTOCENE

Kashmir.—G. S. Puri (Amritsar) is engaged at Lucknow in describing a large collection of fossil remains from the Upper Karewas

of Kashmir. The preliminary results of this study were published in *Proc. Ind. Sci. Congress, Lahore, 1939*, pp. 127-128 and *Ibid.*, *Madras, 1940*, pp. 146-47. Photographs of the genera *Betula*, *Alnus*, *Desmodium*, *Prunus*, *Quercus*, *Sageretia*, *Rhamnus*, *Aesculus*, *Berberis* and *Salix* from this flora, made by G. S. Puri, appeared in the memoir by H. de Terra and T. T. Paterson on the "Ice Age in Kashmir and associated human cultures" (Carneg. Inst. Washington 1939) pls. LIII, LIV. Detailed investigations of C. S. Middlemiss's collections of 1910 from Ludarmarg (Pir Panjal Range) at the altitude of 10,600 ft. (kindly lent to the author by the Director, Geological Survey of India), and H. de Terra's collections of 1932 from the same locality, are in progress. More than 40 species belonging to 30 genera distributed over 22 families of modern plants have been described from leaves, fruits and pieces of twigs. Below are given the genera with the number of species recognised: *Berberis* (2), *Rhamnus* (1), *Berchemia* (1), *Acer* (2), *Desmodium* (4), *Pyrus* (1), *Cotoneaster* (1), *Spiraea* (1), *Parrotia* (1), *Syringa* (1), *Cornus* (1), *Machilus* (2), *Litsea* (3), *Phoebe* (1), *Pittosporum* (1), *Mallotus* (1), *Buxus* (2), *Ficus* (1), *Quercus* (2), *Alnus* (1), *Ulmus* (1), *Skimmia* (1), *Toddalia* (1), *Myrsine* (1), *Ilex* (1), *Trapa* (1), *Typha* (1), *Pinus*, *Taxus*, *Abies*, *Juniperus* and Gramineæ *gen. indet.*

The species of *Quercus*, *Alnus*, *Machilus*, *Litsea*, *Phoebe*, *Mallotus*, *Ficus* and *Acer* found as fossils in this flora do not grow in the Kashmir valley to-day; however, they are common forest trees in nearby regions in the Himalayas.

Quantitatively the best represented genus in the collection is *Quercus*. Such a great abundance (as much as 70%) of oak leaves suggests the probability of oak forest near the site of deposition. The study of this flora promises far-reaching conclusions which are likely to throw light on the Ice Age in Kashmir. Further material from Laridura, Botapathri and Ningal Nullah has been collected by the author this summer.

Ceylon.—G. S. Puri is describing a fossil stem collected by P.E.P. Deraniyagala of the Colombo Museum from a gem pit at Colombagama in Ratnapura district. The greater part of the specimen is a pith cast of *Bambusa* sp. with a clear node and a part of an internode. Portions of the wood with the fibrovascular bundles are also preserved. Macerated preparations have been made from the organic matter still preserved in the specimen with the fossil.

On splitting a portion of the pith-cast, which consisted of a filling of ferruginous rusty brown gravel, a few impressions of dicotyledonous leaves were discovered which seem to belong to species represented in the present-day flora of Ceylon. Leaves of the comparable modern genera are being examined for comparison with the fossil impressions.

PERSONAL NEWS

MISS C. VIRKKI is now Mrs. C. Jacob (see new address below).

NEW MEMBER OF THE COMMITTEE

JANET, M. (MISS), M.Sc., Teacher in Botany, Isabella Thoburn College; Research Student, The University, *Lucknow*, U.P.

NEW ADDRESSES

JACOB, MRS. C. (formerly Miss Chinna Virkki), PH.D., c/o Dr. K. Jacob, Geological Survey of India, *Calcutta*.

PURI, G. S., M.Sc., Research Student, Department of Botany, The University, *Lucknow*, U.P.

RAO, H. S., D.Sc., Fruit Research Station, *Hessarghatta* (Mysore State).

VARMA, S. C., D.Sc., Imperial Agricultural Research Institute, *New Delhi*.

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STUDIES IN ABSORPTION AND TRANSPIRATION*

III. Effects of Hypertonic Solutions on Leaf Turgidity

BY T. EKAMBARAM AND MISS V. K. KAMALAM

Received for publication on September 4, 1940

THE problem of the ascent of sap has been studied by various investigators, but the part played by living cells in this ascent is under dispute. According to Bose,¹ water is pumped by and through pulsating cortical cells in which waves of contraction and relaxation succeed. He was of opinion that the activity of pulsating cells could be interfered with by action of a stimulant or a depressant. If the former acted on the living cells, absorption of water would be increased whereas with the latter it would be the reverse. This view finds only very few supporters.

Strassburger², Overton⁵ and Macdougall showed that the ascent of sap could go on in the plant even after the death of the cells of the stem cortex. Similarly Ekambaram and Rao³ found that the killing of the cortical cells of a cut shoot of *Barleria cristata* with 20% formalin did not cause a change in the rate of absorption. When hypertonic solutions were supplied by them to the cut end of a shoot, they found that there was a fall in the rate of absorption as the solution passed up the stem. The magnitude of the fall depended on the concentration of the solution supplied, age and water content of the shoot. The fall was negligible in the case of an old shoot and did not affect transpiration. But in young and turgid shoots, both absorption and transpiration were affected. This was explained as being brought about by a shrinkage of the cortical cells which cause a mechanical constriction of the vessels, thereby increasing resistance for the passage of water through them. With older shoots, the constriction of the vessels was taken to be less because of their greater rigidity.

In the present study, evidence is offered to show the correctness of the explanation given above by recording the effects of hypertonic solutions on leaf turgidity, when applied to the cut ends of shoots. Incidentally, evidence is offered against Bose's stimulation theory.

APPARATUS

The movement of the leaf was recorded by attaching a lever to a suitable part of it. The change in the position of the lever

* Contribution from the Presidency College Botany Laboratory, Madras.

was recorded on a revolving drum by* burning the paper on the drum at definite intervals by passing an electric spark through it. The whole arrangement is shown in Fig. 1.

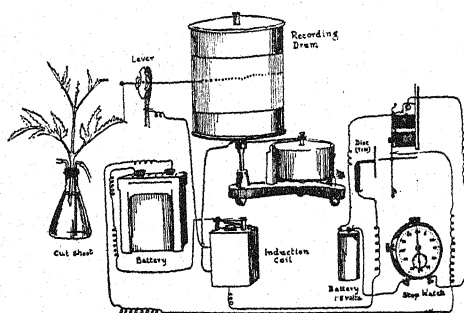


FIG. 1

MATERIALS

The experiments were conducted with young and old shoots of *Tecoma stans*, *Barleria cristata* and *Artemesia* species. The old shoots in the case of *Tecoma* had a mature lower portion which represented the growth of the previous season and a young portion at the top which was formed during the present season.

The usual precautions in the preparation of cut shoots for experiments were observed. Sodium chloride and Potassium bromide were the salts used.

RESULTS

Tecoma stans :

Experiment I—Gr. 9—Effect of 10% Sodium chloride at the cut end.

Nature of the shoot.—Young, three pairs of leaves; cut end to first pair of leaves to which lever was attached 2". Length of the petiole to which the lever was attached 1½".

Recording started with cut end in water 10–15 A.M., change to 10% sodium chloride 10–29 A.M. The leaf was steady for the next two minutes. This was followed by a gradual fall for 5 minutes and then there was a very quick fall. At the end of 17 minutes after treatment the lever had moved to its maximum and recording was stopped.

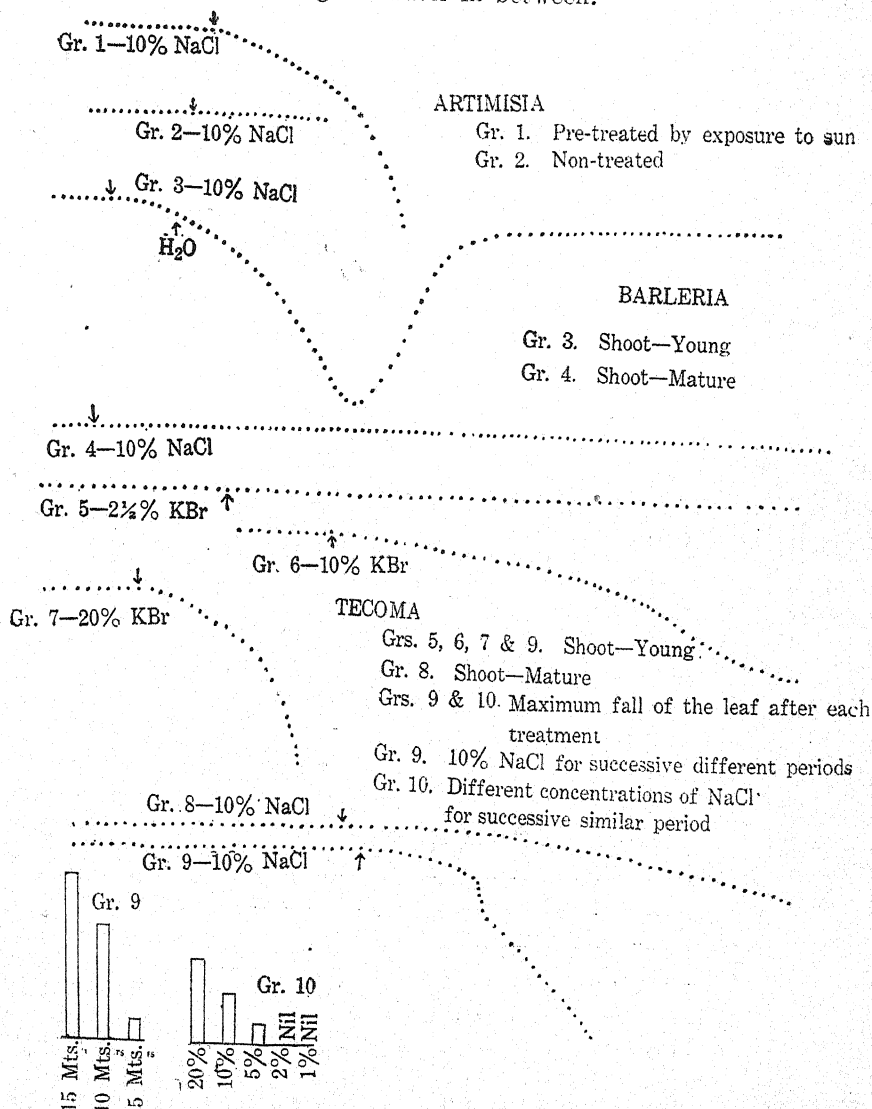
Experiment II—Gr. 8—10% Sodium chloride.

Nature of the shoot.—Old, three pairs of leaves, mature stem 2", young stem 1½"; length of the petiole to which the lever was attached 1¾".

* The graphs presented are direct but reduced reproductions of actual records.

Recording started with the cut end in water 11-55 A.M. Change to 10% NaCl 12-10 P.M. No change in the position of the leaf for the next 7 minutes. Gradual fall started later.

Experiment III—Gr. 10.—10% NaCl supplied with the cut end of the same shoot for successive periods of 15 minutes, 10 minutes and 5 minutes with change to water in between.



Nature of the shoot.—Young, three pairs of leaves; cut end to the first pair $2\frac{1}{4}$ " ; length of the petiole $1\frac{1}{2}$ ".

Recording started in water 8-45 A.M.; change to 10% NaCl 9 A.M.; no change in the position of the leaf for the first 3 minutes and gradual fall from 9-3 A.M. Change to water 9-15 A.M.; fall continued till 9-22 A.M. and then gradual rise. At 10 A.M., recovery to the initial position was completed. At 10-15 A.M. change to NaCl solution. 10-25 A.M. change to water. Fall started at 10-17 A.M. and continued till 10-40 A.M. Recovery started at 10-49 A.M. and was completed by 11 A.M. Change to NaCl solution 11-45 A.M. Change again to water 10-50 A.M. Fall started at 10-47 A.M. and continued for a short period. Recovery was completed by 12-5 P.M.

Maximum fall of the leaf after each treatment with NaCl as magnified by the lever:—

10% NaCl for 15 mins.	Fall 6.5 cms.
" " 10 "	" 4.5 "
" " 5 "	" 0.7 "

Experiment IV—Gr. 11.—Application for 5 minutes in each case of 1%, 2%, 5%, 10% and 20% NaCl to the cut end of the same shoot at successive periods with change to water in between.

Nature of the shoot.—Young, three pairs of leaves; cut end to the first pair 2". Length of the petiole to which the lever was attached $1\frac{1}{2}$ ".

2-30 P.M.—recording started in water.

2-50 P.M.—change to water, no change in the position of the leaf.

2-55 P.M.—change to water, no change in the position of the leaf.

3-5 P.M.—change to 2% NaCl.

3-10 P.M.—change to water, no change in the position of the leaf.

3-20 P.M.—change to 5% NaCl. Fall started after 2 minutes.

3-25 P.M.—change to water. Fall continued.

3-31 P.M.—maximum fall.

3-34 P.M.—recovery started.

3-44 P.M.—recovery completed.

3-50 P.M.—change to 10% NaCl. Fall started after one minute.

3-55 P.M.—change to water. Fall continued.

4-1 P.M.—maximum fall.

4-13 P.M.—recovery started.

4-30 P.M.—recovery completed.

4-30 P.M.—change to 20% NaCl. Fall started in one minute.

4-35 P.M.—change to water. Fall continued.

5-17 P.M.—maximum fall.

6-10 P.M.—no sign of recovery. Recording stopped.

Maximum fall of the leaf after each treatment as magnified by the lever :

1%	Solution of NaCl—5 mins.	Fall nil.
2%	" " "	" "
5%	" " "	" 0.8 cms.
10%	" " "	" 2.0 cms.
20%	" " "	" 3.2 cms.

Experiment V—Gr. 7.—20% KBr.

Nature of the shoot.—Young, three pairs of leaves; cut end to first pair $2\frac{1}{4}$ " ; length of the petiole to which the lever was attached $1\frac{1}{2}$ ".

Recording started in water 8-10 A.M. Change to KBr solution (20%) 8-15 A.M. Fall of the leaf started after one minute. The fall was rapid and reached its maximum by 8-27 A.M. The recording was then stopped.

Experiment VI—Gr. 6.—10% KBr.

Nature of the shoot.—Young, three pairs of leaves; cut end to the first pair $2\frac{1}{4}$ " ; length of the petiole $1\frac{1}{2}$ ".

Recording started in water 9-55 A.M. Change to 10% KBr 10 A.M. Fall of the leaf started after two minutes. Maximum fall was reached by 10-26 A.M. The rate of fall was slower than in the former case (i.e., when treated with 20%).

Experiment VII—Gr. 5.— $2\frac{1}{2}$ % KBr.

Nature of the shoot.—Young, two pairs of leaves; cut end to the first pair 2". Length of the petiole to which the lever was attached $2\frac{1}{4}$ ".

Recording started in water 12 noon; change to $2\frac{1}{2}$ % KBr at 12-10 P.M. No fall of the leaf.

RECAPITULATION OF RESULTS

1. When young and old shoots of *Tecoma stans* were treated with 10% NaCl, the time taken for the leaf fall to commence was considerably less with the young shoot and the rate of fall was also quicker in the young shoot.

2. When the same solution was supplied to the cut end for different periods, the magnitude of the fall depended on the period of supply.

3. When different concentrations were supplied to the cut end for the same period, the magnitude of fall depended on the concentration and the time required for the commencement of the fall was shortest with the highest concentration.

4. When different concentrations of Potassium bromide were supplied, the time required for the commencement of the fall and the magnitude of the fall depended on concentration.

Barleria cristata :

Experiment I—Gr. 3.—10% NaCl.

Nature of the shoot.—4 pairs of leaves; cut end to the second pair 3". Lever attached to second pair.

Recording started in water. Change to 10% NaCl 1-20 P.M. Fall started at once. Change to water 1-28 P.M. Fall continued for the next 26 minutes and then recovery commenced. Recovery was completed 45 minutes after the supply of water.

Experiment II—Gr. 4.—10% NaCl.

Nature of the shoot.—Mature, 5 pairs of leaves.

Recording started in water 2-5 P.M. Change to 10% NaCl 2-10 P.M. No fall in the position of the leaf.

The reaction of a shoot of *Barleria* was similar to that of *Tecoma* when osmotic solutions were supplied to the cut end of the shoot.

Artemesia sp.

Experiment I—Gr. 2.—10% KBr.

Nature of the shoot.—Young, 18 leaves; cut end to the leaf to which lever was attached 5½".

Recording started in water 8-20 A.M. Change to 10% KBr 8-33 A.M. No fall till 8-50 A.M. when the recording was stopped.

Experiment II—Gr. 1.—10% KBr.

Nature of the shoot.—Young, 10 leaves; cut end to the leaf to which the lever was attached 3".

Treatment given to shoot before recording was started.—Cut at 7-50 A.M., exposed to sun 30 minutes, cut again under water and kept in the laboratory with the cut end in water.

Recording started in water 9 A.M., when the position of the leaf was steady; change to 10% KBr 9-16 A.M. Leaf fall started at once and continued for the next 28 minutes, when recording was stopped.

Experiment III.—10% NaCl.

Nature of the shoot.—Young, 6 leaves.

Treatment given to shoot before recording was started.—Cut at 10 A.M., exposed to sun 30 minutes. Leaves wilting; cut again under water and kept with the cut end in water, leaves recovered turgidity within 10 minutes, removed shoot from water at 12 noon and exposed to air in laboratory for 20 minutes, cut under water again and left with the cut end in water. Shoot normal in appearance.

Recording started in water 2-25 P.M., leaf steady. Change to 10% NaCl 2-32 P.M. Fall commenced after one minute and continued at a rapid rate (no graph presented).

Experiments similar to Experiment I but with 10% NaCl instead of KBr gave similar results. When a young shoot of

Artemesia, cut and kept under water, was later changed to 10% KBr or NaCl, the leaf of the shoot showed no leaf fall for the first half an hour, but when the same shoots were treated first in such a way as to bring on a water deficit, without wilting, and were subsequently treated with the same osmotic solutions, fall in the position of the leaf occurred even within a few minutes.

A large number of experiments similar to those described above but using different osmotic solutions such as KNO_3 , sugar, etc., gave parallel results.

DISCUSSION AND CONCLUSION

The experiments described with young shoots of *Tecoma stans* and *Barleria cristata* show that in the case of young shoots of these plants there is a fall in the position of the leaf very soon after the application of a hypertonic solution to the cut end of the shoot. The fall in the leaf is an indication of the loss in the turgidity of the leaf cells due to transpiration being greater than water supply. Ekambaram and Rao⁴ have shown that under similar conditions both absorption and very soon transpiration show a fall. As long as the shoots are in water, the balance between supply and loss is kept up. But the hypertonic solution at the cut end has caused a disturbance in the water balance of the cut shoot. If the behaviour of a young shoot is compared to that of an old one under similar conditions, it is seen that the leaf fall is very much delayed in the older shoot. This indicates that in the old shoot, the leaf fall begins only when the hypertonic solution reaches the young portion of the stem. This difference between the young and the old shoots can be accounted for by the difference in the nature of the xylem in the two. In the older part of the shoot, the wall of the xylem vessel will be more rigid and less compressible than the walls of the vessels in the younger parts. When the tensions in the stem tissues are altered by the entry of the highly osmotic solutions, the vessels in the young shoots get compressed and offer resistance to the upward flow of the solutions and the water balance in the leaf is quickly affected.

The other experiments described with *Tecoma stans* go to confirm the explanation given above. Pierce,⁶ after describing the effects of freezing on water conduction, comes to the conclusion that the living cells of the stem tissues condition the ascent of sap, but that they cannot be said to cause it. The present study shows how the conditioning is brought about.

The experiments with *Artemesia* show that even with young shoots the water balance in the leaves is an important factor. It is seen that when the leaf cells are fully turgid, the loss of water required to bring on drooping of the leaf has to be considerable. This is why the response of an *Artemesia* shoot to the hypertonic solution is different from that of *Barleria cristata* or *Tecoma stans*. But when the water balance in the leaf is adjusted by pretreatment then the response is similar.

Bose,² in his experiments for showing pulsation stimulation of cortical cells, always used *Chrysanthamum* shoots which had been partially desiccated by previous treatment. It is evident that response of the nature attributed by him to stimulant or depressent could not have been obtained with untreated fresh shoots.

SUMMARY

1. Experiments are described for measuring the initiation and progress of loss of turgidity in the leaves of cut shoots of *Tecoma stans*, *Barleria cristata* and *Artemesia* sp., as indicated by change in position of the leaf.

2. When hypertonic solutions are applied to the cut ends of young shoots of *Tecoma* and *Barleria* there is a very quick loss of turgidity in the leaf cells and the leaf falls. The fall is delayed in mature shoots till the solution reaches the young part of the shoot. The explanation is offered that in young shoots a constriction of the vessels is brought about.

3. In the case of *Artemesia*, if the leaves are fully turgid no fall occurs for quite a long time. But if the water content of the shoot is reduced by previous treatment, there is a quick fall as in the previous cases. The depressive response of Bose is considered to be really due to the constriction of vessels in the stem.

Part of the work of this paper was carried out in collaboration with Mr. I. N. Madhusudana Rao, M.A., M.Sc., and our thanks are due to him.

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STUDIES IN ABSORPTION AND TRANSPIRATION*

Part IV. The Effect of Oxygen concentrations on absorption of water and transpiration

BY MISS V. K. KAMALAM

(Communicated by T. Ekambaram)

Received for publication on September 4, 1940

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INTRODUCTION

WATER is one of the important factors that control plant growth and plant yield. The main source of water for a plant is the soil. A study of water absorption by the roots is therefore of great importance both from the point of view of plant physiologists and agronomists. In spite of its importance, the problem has not received as much attention from botanists as it should. We have however a limited knowledge of some of the factors that influence the entry of water into the roots—as temperature, osmotic concentration, pH value of the solution surrounding the root, effect of specific ions, etc. But our present-day knowledge of the importance of soil gases in absorption is very meagre. The influence of soil aeration on root growth has been emphasised by various workers; but very little work has been done so far to show the effect of these gases on absorption of water. This study was therefore undertaken with the view of finding out the effects of soil gases on absorption of water by the roots. Oxygen and carbon-di-oxide which are supposed to exert considerable influence on root growth and root activities have been taken separately and their individual effects on absorption of water have been studied. The present paper deals with the effect of oxygen concentrations on absorption. The effects of carbon dioxide concentrations on the same physiological processes will be published later.

* Part of the thesis approved for the M.Sc. Degree of the Madras University. Contribution from the Presidency College Botany Laboratory, Madras.

MATERIALS AND METHODS

For the preliminary experiments, a number of plants were tried like Balsam, *Amaranthus*, Tomato, *Erythrina*, Beans, Cotton, and *Polygonum*. Of these, *Erythrina indica* and *Polygonum glabrum* were finally chosen for the present investigation because of the comparative ease with which they could be grown in water culture.

In the case of *Erythrina indica*, the seedlings were grown in Knop's solution from the germination stage. Cuttings of *Polygonum glabrum* were grown in ordinary tap water. In both cases, plants made normal growth of the shoot and the root systems.

*Selection of Plants for Experimentation**Erythrina indica*—

In spite of all precautions taken in selecting the seeds and growing them under similar conditions, the plants did not exhibit uniformity of growth. For all experimental purposes, plants having similar appearance of the shoot and the root were selected. The selection was however confined to individuals between 14 to 18 days old. In all cases detailed records of the shoot and the root were kept along with the exact age of the plant so as to make the results strictly comparable.

Polygonum glabrum—

For any one set of experiments done on the same day, plants of the same age and similar appearance were selected.

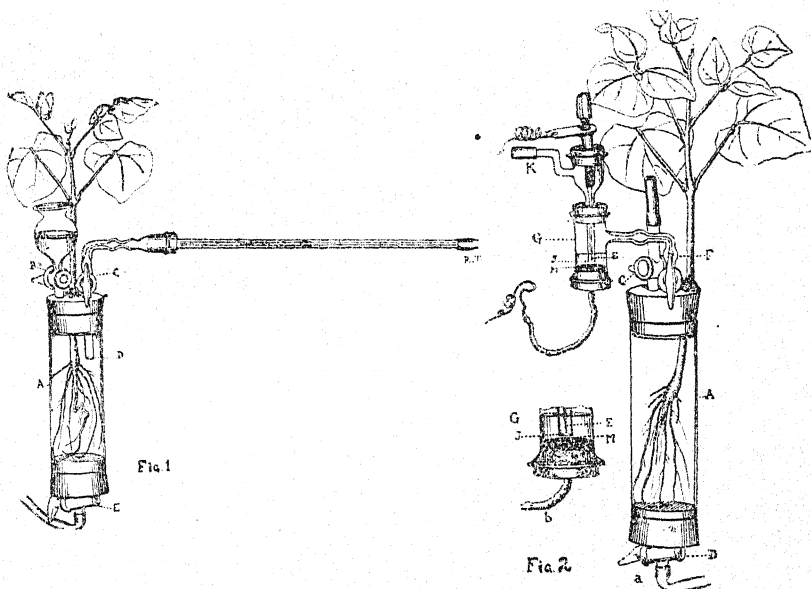
Apparatus—

Absorption of water was measured in two ways :—

1. Direct (Potometer) method
2. Automatic recording method

1. *Direct Method*—This method is very simple and has been adopted by a number of workers to measure absorption. Tube A (Fig. 1) of 80 to 100 c.c. capacity was first filled with distilled water. A three-holed rubber stopper was then gently pushed in. One of the holes took the plant and the other two had two tubes with stop-cocks B and C passing through them. The upper end of B was blown out into a bulb which served as a water reservoir. C terminated in a graduated pipette (2 c.c. divided into 200 divisions). After pushing in the stopper with tubes, the whole arrangement was made air-tight and completely filled with water. The end D of stop-cock C always remained under water inside A. To the end of the graduated pipette was attached a rubber tubing RT. The whole arrangement was then immersed in a water-bath, in such a way that only the tip of the rubber tubing and the neck of the reservoir were above the water-level. The region of the stem under water was coated with paraffin of low melting point so as to avoid contact with water. By closing C and by opening B and E, water in the tube could easily be changed without disturbing any of the external conditions. The

reservoir was always filled with the same solution as in A. After introducing the solution into the bulb, a thick film of paraffin oil was poured over the surface and on this again, was poured hot paraffin, which on cooling formed a thin crust. These precautions were taken to minimise the diffusion of gas from the solution in the reservoir.



Readings were taken at fixed intervals (ordinarily every half hour) and the quantity of water absorbed by the plant was noted directly by reading the movement of the meniscus in the graduated tube. Whenever needed, the stop-cock B was opened and water was let in from the reservoir to the tube A and the meniscus brought to the desired position in the graduated tube.

2. *Automatic Recording Method*—A few preliminary experiments were first done with the automatic micro-absorption apparatus used by Ekambaram and Rao (1933). Later it was found desirable to modify the lever part of the apparatus. A new device was therefore adopted, the principle of which is similar to the method employed by Knight (1922) to measure stomatal apertures.

Description and Manipulation of the Automatic Apparatus—As in the Potometer method here also tube A was first filled with distilled water (Fig. 2). The three-holed rubber stopper with the plant and the glass tubes was then gently pushed in. The whole arrangement was later made air-tight, special care being taken to seal both the ends of the tube G. The apparatus was then placed in a water-bath. By manipulating F, C and D, water from the tube A could be siphoned out and any desired solution put in without altering any of the external conditions. The stop-cocks C and D

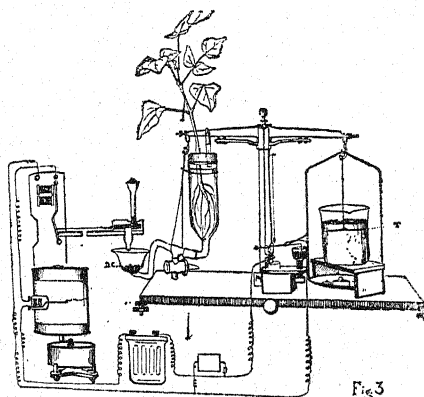
were then closed and F opened and the rate of absorption again recorded.

As the plant absorbed water, the pressure in tube A was reduced. Air was drawn through K and finally bubbled out through water in tube G. As the bubble was formed at the tip of J, it depressed the mercury surface M and when the bubble escaped the mercury surface oscillated slightly and made a momentary contact with electrode E. This completed the circuit and a record was made on the revolving drum.

The volume of water absorbed per bubble was measured by attaching a calibrated tube to tube K. A small drop of oil was sent into this tube from the open end. The movement of oil in the tube was noted for each bubble. Averages of a few readings were taken as the volume per bubble.

The bubbler, when once adjusted, showed no variation in the volume of the bubbles and the manipulation of the other parts of the apparatus caused no disturbance in the bubbler. The volume per bubble was ordinarily adjusted to 0.02 to 0.04 c.c. With this volume the rate of absorption was from 5 to 20 bubbles per 15 minutes depending on the age of the plant and the external conditions.

The important precaution that had to be taken in the case of the automatic recording method was the adjustment of the distance between the mercury surface and the end of the tube J. If this distance was too little then bubbling never gets uniform, as the pressure inside was never equalised fully, when the bubble escaped into the root chamber.



Transpiration—

The method employed by Ekambaram and Rao (1933) was adopted to measure the transpiration of plants (Fig. 3). This method was found to be very efficient and simple. The transpiring plant was hung from one end of the beam of a balance. Weights were added to the other pan so as to bring the beam into equilibrium. When the plant lost a certain definite weight of

water, a steel ball was automatically dropped into (Dummy cup) D.C. of the apparatus which was not in connection with the root chamber. This made a mark on the revolving drum.

Experimental Procedure—

The palnts selected for the experiments were brought up from the green house the evening previous to the day of experimentation, fitted up in the tubes filled with tap water, after washing the roots thoroughly, and left outside in the laboratory. The next morning the tap water was changed with distilled water exposed to air, the apparatus fitted up and placed in the water-bath for about two hours before the recording was started.

During the period of experimentation the external conditions which affect the transpiration of the shoot were not kept under control. But the variations were of a small magnitude since the apparatus was kept in a protected room. A number of control experiments done during the year of 1935-'36, 1936-'37, 1937-'38 showed that absorption of water by the roots kept steady throughout the experimental period during each day from the end of May till the beginning of July. A control experiment was found to be unnecessary at that part of the year although now and then they were run as checks.

During the other parts of the year, particularly, in August and September, when the rains commence in Madras, a control was found to be absolutely essential.

Often more than two plants, in addition to the control, were fitted up. These received uniform treatments on most of the days so that any difference, in individual behaviour could be noticed. Usually they were fitted up one immediately after the other.

General Methods of Treatment—

Two sets of treatments were given.

I. Treatment for the First Lot—

TREATED	CONTROL
8 A.M. Tap water to distilled water. Apparatus placed in the water-bath.	8 A.M. Tap water to distilled water. Apparatus placed in the water-bath.
11 A.M. Change to oxygenated water.	11 A.M. No change.

Later on, a slight alteration was introduced in the mode of treatment, for reasons discussed under results.

II. Treatment for the Second Lot—

TREATED	CONTROL
8 A.M. Tap water to distilled water. Apparatus placed in the water bath.	8 A.M. Tap water to distilled water. Apparatus placed in the water-bath.
10 A.M. Change to fresh d'stilled water. Recording started.	10 A.M. Change to fresh distilled water. Recording started.
11 A.M. Distilled water to the desired solution.	11 A.M. Distilled water to distilled water.

In the potometer and transpiration experiments, the second procedure was adopted.

Presentation of Data—

Even though care was taken in selection of plants to give uniformity, the absorption or transpiration of individual plants were not comparable with each other. To make comparison of the different experiments possible, the method described here, of presenting the data, was followed.

The number of bubbles recorded during the last fifteen minutes before the treatment, was taken as the Initial Rate. The number of bubbles for every fifteen minutes after treatment was then calculated as percentages in terms of the initial rate and the graphs plotted.

The same method was adopted for plotting the graphs of the potometer and transpiration experiments. In the case of the former the readings were taken every half hour. The absorption for every fifteen minutes period, was then calculated from the half hour so as to make the graphs of the Automatic and Potometer Methods comparable. The method of plotting the graphs in terms of the initial rate was followed.

In the case of transpiration experiments, the quantity of water lost every one hour was first noted and from it the loss for every fifteen minutes was calculated. Graphs were plotted as in the other cases.

The data of three typical experiments (two on absorption according to the two methods and one on transpiration) are given below. The first of these experiments show the detailed manner in which the records of the root system have been kept.

*Direct Method (Potometer)—**Experiment I. (8-9-38). Table I.*

Effect of 18.5 mgms. per litre dissolved oxygen on absorption of water by the intact roots of *Erythrina indica*.

First Change. Treated 10-5 to 10-7 A.M. Control 10 to 10-5 A.M.

Second Change. Treated 11-35 to 11-38 A.M. Control 11-30 to 11-35 A.M.

TABLE I
Expt. I. Direct Method

TREATED			CONTROL			Air temp. °C.	REMARKS
Quantity absorbed			Quantity absorbed				
30 mts. (recorded) c.c.	15 mts. (calculated) c.c.	% of initial rate	30 mts. (recorded) c.c.	15 mts. (calculated) c.c.	% of initial rate		
0.6175	0.3088	95	0.2800	0.1400	84	30.75	Day bright
0.6650	0.3325	102	0.3075	0.1538	93	30.75	do.
0.6500	0.3250	100	0.3325	0.1663	100	31.75	do.
CHANGE			CHANGE				
0.6950	0.3495	108	0.3550	0.1775	107	32.0	do.
0.6175	0.3088	95	0.3400	0.1700	102	32.0	do.
0.6175	0.3088	95	0.3550	0.1775	107	32.0	do.
0.6950	0.3475	108	0.3600	0.1800	108	32.0	Slightly cloudy
0.6000	0.3000	92	0.3250	0.1625	98	32.0	Very cloudy
0.5450	0.2725	84	0.2605	0.1303	78	32.0	Slightly drizzle
0.5450	0.2725	84	0.2200	0.1100	66	31.75	Clearing

Temperature of the water .. 30.75°C. \pm 0.5°C.

Description of the Plants used for Experiment I.

TREATED	CONTROL
Shoot 3" tall (4 leaves)	Shoot 7" tall (4 leaves)
Roots healthy and white but very thin.	Roots healthy and white.
Tap root 3" long.	Tap root 3.5" long
2 lateral roots 2.5" long (2 br. $\frac{1}{2}$ ")	1 lateral root 3.5" long (2 br. 1")
5 3" .. (2 br.)	1 4.0" .. (5 br. $\frac{3}{4}$ ")
30 2.0" .. (6 br. $\frac{1}{4}$ "- $\frac{1}{2}$ ")	2 3.0" .. (5 br. $\frac{1}{4}$ ")
5 2.5" .. (no br.)	2 2 $\frac{1}{2}$ " .. (no br.)
1 3.5" .. (11 br. 1")	7 1 $\frac{1}{2}$ " .. (..)
2 3.0" .. (5-6 br. $\frac{1}{2}$ ")	8 2" .. (..)
28 2.5" .. (no br.)	4 3 $\frac{1}{2}$ " .. (..)
	13 2" .. (..)
	14 1 $\frac{1}{2}$ " .. (..)
	7 1" .. (..)
	1 2" .. (5 br. $\frac{1}{2}$ ")

*Automatic Recording Method—**Experiment II. (3-8-38). Table II.*

Effect of 19 mgms. per litre of dissolved oxygen on absorption of water by the intact roots of *Erythrina indica*.

Set up at 8 A.M.

First Change. Treated 10-8 to 10-10 A.M. Control 10-5 to 10-8 A.M.

Second Change. Treated 11-7 to 11-10 A.M. Control 11-15 to 11-20 A.M.

TABLE II

Expt. II. Automatic Recording Method

TREATED		CONTROL	
No. of bubbles per 15 mts. (recorded)	Percentage of initial rate	No. of bubbles per 15 mts. (recorded)	Percentage of initial rate
18	90	18	113
22	110	18	113
21	105	16	100
20	100	16	100
CHANGE		CHANGE	
21	105	18	113
21	105	16	100
21	105	16	100
20	100	17	106
19	95	16	100
20	100	16	100
18	90	16	100
18	90	15	94
18	90	15	94
18 [*]	90	16	100
20	100	16	100
16	80	15	94
16	80	16	100
17	85	15	94
16	80	15	94
14	70	14	88

Volume of the bubble .. 0.03 c.c.
 Air temperature at 11-15 A.M. .. 32°C.
 Water temperature at .. 30.75° C.
 Day bright throughout.

*Transpiration—**Experiment III.* (5-9-38). Table III.

Effect of 22 mgms. per litre of dissolved oxygen on transpiration of *Erythrina indica*.

Second Change. Treated 11-30 to 11-40 A.M. Control 11-40 to 11-55 A.M.

TABLE III
Expt. III. Transpiration

TREATED			CONTROL		
Balls per hour	Balls per 15 mts. (calculated)	Percentage	Balls per hour	Balls per 15 mts. (calculated)	Percentage
50	12.5	100	11	2.75	100
	CHANGE			CHANGE	
55	13.75	110	15	3.75	136
65	16.25	130	16	4	145
70	17.5	140	16	4	145
65	16.25	130	14	3.5	127

Air temperature at 11 A.M. . . 32.25 °C.

Day clear and bright till 12-20 P.M. Slightly cloudy at 12-20, but cleared soon. Again cloudy at 2 P.M. and continued to be so till the end of the experiment.

In order to save space, the data of the various experiments and the details of the root systems have been omitted from this paper. The results will therefore be presented only in the form of graphs.

Method of Preparing Solutions—

Oxygen from a cylinder (gas from the cylinder was analysed to make sure that there was no CO₂ in it) was bubbled through distilled water in a bottle with an inlet and an outlet. Bubbling through for different periods gave different concentrations of oxygen in the solution. This bottle was then stoppered air-tight and immersed in the water-bath at least one hour before the period of experimentation to equalise its temperature with that of the water in the bath. After transferring the required amount of solution to the root chamber, the rest of it was used to estimate the quantity of dissolved oxygen by the Winkler Method.

RESULTS

Absorption—

Before a critical study of the graphs are made, the two methods of treatment preparatory to the introduction of oxygen solution into the root chamber as described under methods, require explanation.

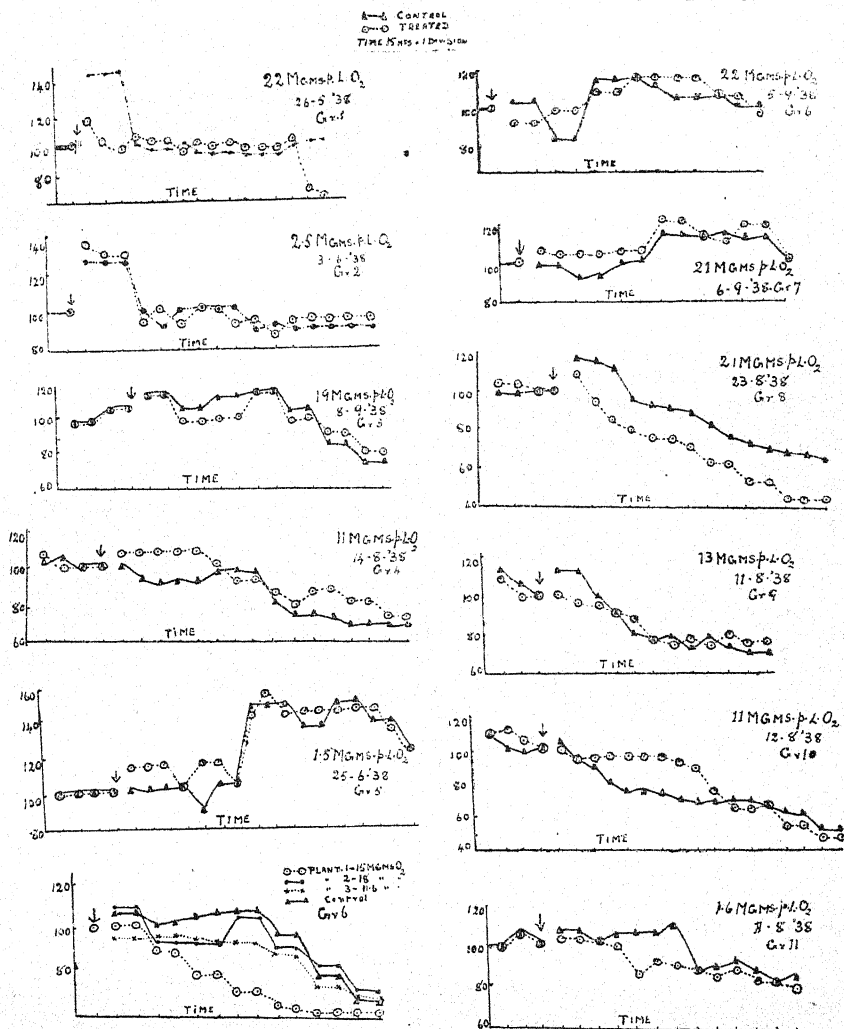
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During the early period of this work, the first method of one change was adopted. Graph Nos. 1 and 2 would show that though the oxygen concentrations varied, the first part of the graph in all the treated cases was similar and showed a sudden rise immediately after treatment. This indicated a factor independent of the oxygen concentrations of the solution introduced. Therefore experiments were done to eliminate this factor.

ABSORPTION

ERYTHRINA INDICA

POLYGONUM GLABRUM



A number of experiments were then carried out to see the effect on absorption of changing the water in the root chamber. Finally

it was found that a change of water, one hour before the introduction of oxygen solution, would eliminate this initial rise. The second method of treatment described was therefore adopted in all the other experiments.

The oxygen concentrations used in the present study ranged over wide limits. The exact concentrations used fell under the following groups:—

I.	15–23 mgms. per litre of oxygen dissolved in water	<i>Erythrina</i> Grs. 1, 3 and 6 <i>Polygonum</i> Grs. 6, 7 and 8
II.	10–15 mgms.	Do.	..	<i>Erythrina</i> Grs. 4 and 6 <i>Polygonum</i> Grs. 9 and 10
III.	1–10 mgms.	Do.	..	<i>Erythrina</i> Grs. 2 and 5 <i>Polygonum</i> Gr. 11.

Now it is essential to consider the range of individual variations in these experiments before one interprets the results obtained. The results of the three potometer experiments on 8–9–38 (Gr. 6) are of special interest from this point of view. The plants used for these experiments were all pre-treated in the same way and the experiments were conducted on the same day under similar conditions. The treatments given were different in the three cases. For plant 1 (Gr. 6) the concentration of oxygen employed was 15 mgs. per litre while for plants 2 and 3 the concentrations were 18.0 and 11.6 mgs. per litre respectively. The curves for absorption of the treated and the control plants were practically alike in plant 2, but for plant 1 the treated curve was at a lower level than the control. Plant 3 gave an intermediate curve with the absorption for the treated at a lower level than the control, but higher than that of plant 1.

A comparative study of a number of treatments—with 15–23 mgms. per litre oxygen—on *Erythrina* (compare Gr. 6, and Grs. 3, 2 and 1) showed that the curve (Gr. 6, Plant 1) obtained in this case was not typical. The majority of curves obtained under this treatment were similar to the graph of plant 2 as shown in Gr. 6. The differences seen in Gr. 6 therefore must be taken only as individual variation. A number of experiments similar to those referred to here, showed the possibility of individual variations occurring within certain limits. This was found to hold good in the case of *Polygonum* also. Similar variations could be seen in transpiration as well (Grs. 12 to 23).

The graphs obtained under the different groups of oxygen treatments did not show any characteristic differences, taking into consideration the range of individual variations. In all cases, both in *Erythrina* and in *Polygonum* the treated and the control curves showed parallelism. Grs. No. 6 and 7 for *Polygonum* are of special interest. The cuttings in these cases had roots which were

only* three days old. These roots at the time of experimentation were in an actively growing state, with plenty of long and healthy root hairs, which were visible even to the naked eye. As could be seen from the graphs, the treatment gave no difference in the rate of absorption, even though the roots were decidedly younger than in the previous cases.

Transpiration.—

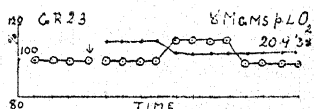
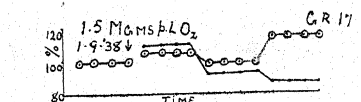
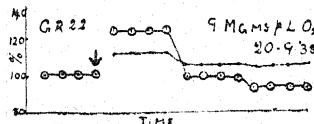
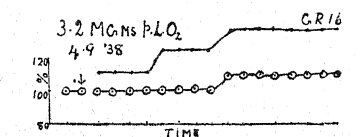
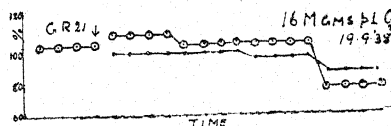
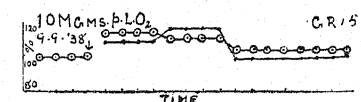
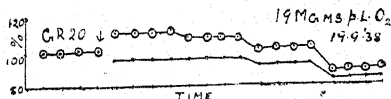
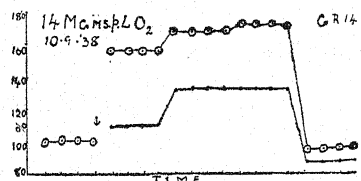
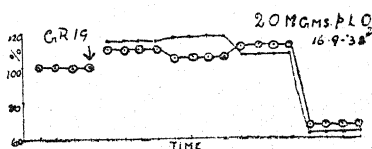
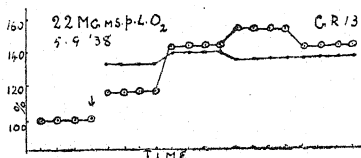
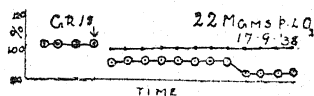
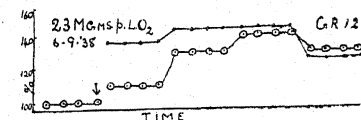
Grs. 12-23 show the effect of oxygen on the transpiration of *Erythrina* and *Polygonum*. In nature, transpiration of plants is

TRANSPIRATION

ERYTHRINA INDICA

POLYGONUM GLABRUM

—○— CONTROL
—●— TREATED
TIME—HOURS—MINUTES



* Age of the roots used ordinarily for the experiments (*Polygonum*) 10 to 14 days.

much more quickly affected by changes in the external conditions than absorption. Though in the present set of experiments no special attempt was made to control the external conditions, yet sufficient care was taken to keep the plants in a well protected room, where sudden changes of external conditions were not likely to happen. Grs. 12-23 show that a certain amount of individual variation exists in the case of transpiration just as with absorption. Apart from these individual variations, the transpiration of *Erythrina* and *Polygonum* were not affected by the oxygen concentrations of the solutions supplied to the roots.

DISCUSSION

So far only very little experimental evidence is available to show the effect of oxygen on absorption of water by the roots. In 1897 Kozarov concluded from his experiments that lack of oxygen lowers the rate of absorption. Maximov cites the results of Kozarov in the following manner:—

“Kozarov experimented with rooted plants in a potometer. By passing a stream of hydrogen or carbon-di-oxide, he deprived the roots of oxygen and was able to observe a delay in absorption. The retarding influence with carbon-di-oxide was more marked than with hydrogen while prolonged exposure to carbon-di-oxide led to the poisoning and dying of the roots. From this Kozarov concluded that hydrogen merely deprives the root of oxygen, while carbon-di-oxide acts as a specific poison.” The present writer was not able to get at the original paper of Kozarov.

Livingston and Free (1917) state, “in the case of those plants which are injured by deficient soil oxygen, it is interesting physiologically, that the first effect of oxygen deprivation is an interference with the absorption of water by the roots.” These investigators experimented with plants grown in soil. Water was supplied to the roots by means of Livingston's Auto-irrigator and absorption was measured by noting the quantity of water lost from the Auto-irrigator.

They found that absorption nearly stops within 24 hours, if Nitrogen was passed through the soil in which *Coleus* and *Heliotropium* were grown. According to these authors, the deprivation of oxygen first affects the respiration of the protoplasm of the absorbing root cells, resulting in their death and coagulation. The lowered rate of absorption was considered by them to be the result of this protoplasmic coagulation and of its subsequent incapacity to function as water absorber. These authors, however, did not present any data in their paper although they had stated their general conclusions.

Bergman (1920) investigated the effect of submergence of the roots on transpiration. The general inference of his study was that

transpiration is increased on the first day after submergence and was soon followed by a fall. The rate of transpiration on the subsequent days was at a lower level as compared with the control.

Hunter and Rich (1925) recorded an accelerated transpiration rate, if air was passed through soil in which *Impatiens balsamina* grew. They did not present any data or graphs to substantiate their statement on this point.

Cannon, Demaree and Purer (1933) studied the relation between evaporation, transpiration and oxygen consumption by the roots. They subjected their plants to sudden changes of light intensity and measured the transpiration of the plant and oxygen consumption by the roots during the dark and the lighted periods. The evaporating power of the air was measured by means of atmometers. They found that a sudden transference of the plant from the dark to the light always interfered with the amount of oxygen used by the roots. A fall in oxygen consumption by the roots was noticed when the plants were suddenly changed from darkness to light. The rate of transpiration, however, increased during the lighted period as compared with the dark. These authors, therefore, concluded that there was no positive relation between the rate of transpiration and oxygen consumption by the roots.

The results obtained by Leta Henderson (1934) were not entirely in agreement with those of Cannon, and others. She found a correlation between the absorption of water and respiration. This investigator enclosed the shoot of the experimental plant in glass chambers and controlled transpiration and thereby absorption, by passing streams of dry and moist air through the shoot chamber. Absorption was measured by direct potometer method and respiration by quantity of carbon di-oxide given off by the roots. In a few cases, respiration was also measured from the quantity of oxygen consumed during unit time. Whether respiration was measured by calculating the oxygen used up or the CO_2 given off, she found a direct correlation between the processes of absorption and respiration.

The extensive work of Cannon showed that different plants responded differently to oxygen treatments—some being very sensitive to oxygen deficiency, while others lived in the absence of oxygen for a number of days. During the years 1936 to 1937, the present author grew seedlings of *Erythrina* for more than two weeks without any direct supply of oxygen to the roots. The plants showed a fairly good amount of both shoot and root growth. These facts show that *Erythrina* is not an oxygen sensitive plant. Since no such experiments were done with *Polygonum*, it is not possible to say whether this plant in turn is oxygen sensitive or not. The present study shows that within the limits tried (1.5 to 22 mgs. per litre) oxygen is not a limiting factor in the absorption of water and transpiration of *Erythrina* and *Polygonum*, during short periods.

In none of the present set of experiments was oxygen at complete deficiency. Whether a further lowering of oxygen below 1.6 mgs. per litre will have any effect on absorption or transpiration is a matter for further investigation.

A comparative study of the results obtained with the two types of plants, *Erythrina indica* and *Polygonum glabrum* are of interest both from the ecological and physiological point of view. The plants investigated grow in entirely different habitats. *Polygonum glabrum* is a herbaceous plant, which usually grows in shallow waters or moist soil, in large clumps, occupying wide areas. The roots are short and occur below the soil or water surface and must naturally be accustomed to a high content of oxygen in water under atmospheric conditions. *Erythrina* on the other hand is a perennial tree with a deep root system.

The similarity in the oxygen effects noted in the case of both these plants throw doubt as to the importance of oxygen concentrations on absorption of water in general. From the present study it appears that a very wide range of oxygen concentrations in the soil water surrounding the roots has practically no influence on either water absorption or transpiration of plants of different habitats.

This is certainly at variance with the generally accepted view of the importance of aeration on plant growth. It is evident from these results that some factor other than pure oxygen concentration has to be looked for, in explaining the undoubted beneficial effects observed when roots are aerated.

SUMMARY

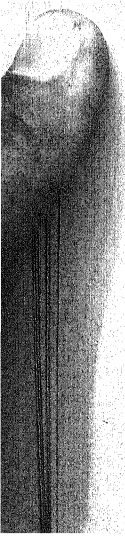
1. The effect of different oxygen concentrations were studied on absorption of water by the roots and transpiration by different methods.
2. The oxygen concentrations ranged from 1.5 to 2.3 mgs. per litre.
3. These concentrations had no specific effect on absorption of water or transpiration.

ACKNOWLEDGEMENT

I take this opportunity to acknowledge with gratitude my deep indebtedness to Dr. T. Ekambaram, M.A., Ph.D. (Cantab.) for his constant guidance and helpful criticism throughout the progress of this investigation, and thank the University of Madras for the award of a studentship during 1936-38 when the major portion of this work was done.

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A MORPHOLOGICAL AND CYTOLOGICAL STUDY OF *POLIANTHES TUBEROSA* LINN.

BY A. C. JOSHI AND J. V. PANTULU

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INTRODUCTION

Polianthes tuberosa Linn., the tuberose, is one of the commonest and most delightful plants in the Indian gardens. In suitable soil it produces tall spikes bearing hyacinth-like clusters of pure white flowers, which diffuse an exquisite fragrance for a wide distance around. Both single and double-flowered varieties are found under cultivation. Of these, the first is more fragrant. The plants are propagated by bulbs. At Benares they flower nearly throughout the year, but more abundantly during the rainy season and in the early part of winter. No seeds are formed, though some of the ovaries during the period of luxuriant growth are quite commonly seen to enlarge considerably after the other parts of the flowers have withered away. The species is unknown in the wild state,

but has been long under cultivation both in the Old and the New Worlds. The genus *Polianthes*, however, includes about a dozen other species. These are found in Mexico, other Central American countries and Trinidad.

A morphological and cytological investigation of *Polianthes tuberosa* is desirable on account of several reasons. Although it has been so long and so much cultivated in all parts of the world, it has received scant attention both from morphologists and cytologists. The number of chromosomes has been reported by Whitaker (1934), but the detailed cytology is unknown. Similarly there has been no work either on the anatomy of the flower or embryology, except a small note by Palm (1920) on pollen formation. Other species and genera of the tribe Poliantheæ, in which this plant has been placed by Hutchinson (1934), are completely unknown both from morphological and cytological stand-points. Further the systematic position of the genus is now a subject for discussion. It has to be decided whether the group Poliantheæ should continue to form a part of the tribe Agavoideæ, as believed by Pax and Hoffmann (1930), Wettstein (1924) and many others, or should be placed in a separate tribe as proposed by Hutchinson (1934). Next, should the tribe Agavoideæ continue to be placed in the Amaryllidaceæ, the position assigned to it in the 'Pflanzen-familien' (Pax and Hoffmann, 1930) and 'Genera Plantarum' (Bentham and Hooker, 1862-83), or should it form a separate family with the Dracenoideæ as suggested by Hutchinson (1934)? Lastly the origin of *Polianthes tuberosa* is uncertain and it would also be worth while to investigate the cause of the marked sterility in the species.

PREVIOUS WORK

The tribe Poliantheæ of Hutchinson (1934) includes three genera, *Polianthes*, *Prochnyanthes* and *Pseudobravia*. Absolutely no morphological and cytological work has been done on the last two genera. The genus *Polianthes* has been investigated by Palm (1920) and Whitaker (1934). Palm showed that pollen grains in *Polianthes tuberosa* are formed in a successive manner, while Whitaker (1934) counted from meiotic divisions in the pollen-mother cells of the same species that the haploid number of chromosomes is thirty, of which five are large and twenty-five small.

The literature on the related tribe Agaveæ, which includes the genera *Agave*, *Furcraea*, *Beschorneria* and *Doryanthes*, is more extensive. Osterhout (1902) studied the development of spindle in *Agave americana*. Lary de Latour (1908) observed karyomery in *Agave attenuata*. Schaffner (1909) investigated meiosis in the pollen-mother cells of *Agave virginica*. He reported 12 haploid chromosomes of unequal size. Muller (1912) reported 20 large and numerous small diploid chromosomes in *Agave americana*, 12 haploid and 24 diploid chromosomes in *A. virginica* (?) and 10 large and 50 small diploid chromosomes in *Beschorneria superba*. From recent work it is clear that the reports of both Schaffner and Muller about the

chromosome number of *Agave virginica* are incorrect. Further what Muller regarded as *A. americana* was probably some other species. Ernst (1918) investigated the development of the embryo-sac and pollen in *Furcraea cubensis*. He found the ovules bitegmic, formation of a wall cell, four megaspores, Normal-type of embryo-sac and nuclear endosperm. Schlimbach (1924) in his morphological study of the ovule and seed of Amaryllidaceae investigated *Agave chlorantha*, *A. attenuata* and *Furcraea altissima*. He found in all the three plants ovules bitegmic and four megaspores. In the last species he observed embryo-sac haustoria penetrating the nucellus and pollen unable to germinate. Heitz (1926) reported ca. 40 and ca. 50 as diploid chromosome number in *Furcraea Lindenii* and *F. altissima* respectively, of which 10 were found to be large in both species. It is probable that his counts of small chromosomes are incorrect and the total number in both species is 60. Nevins (1927) described the development of the female gametophyte of *Furcraea Andina*. Newman (1928 and 1929) gave a very full account of all phases of the life-history of *Doryanthes excelsa*. The formation of pollen grains corresponds to the Simultaneous-type. Mature pollen grains are 2-nucleate. The generative cell precedes the tube nucleus during development of the pollen-tube. Development of the embryo-sac agrees with the Normal-type. The synergids have prominent filiform apparatus. The antipodals are small, persistent and sometimes multiply in number. Development of the endosperm corresponds to the Helobiales-type. Chromosome number is 18-22 haploid. Catalano (1929 and 1930) made embryological and cytological studies in the genera *Agave* and *Furcraea*, reporting 7 as the haploid chromosome number in *Agave Sisalana* and 9 as the haploid number in *Furcraea gigantea*. Both these counts are clearly incorrect. Koerperich (1930) reported 60 diploid chromosomes in *Beschorneria Yuccoides*. Catalano (1931a) investigated the development of embryo-sac and pollen in *Agave atrovirens* with special reference to the partial sterility of many individuals. He concluded that this is influenced by nourishment. Even absolutely sterile plants show pollen grains which can germinate in artificial sugar cultures and embryo-sacs which do not at all degenerate till the time of fertilisation. On the other hand even in highly fertile individuals always only a small percentage of ovules in an ovary form seeds. The same author (Catalano, 1931b) gave an account of the morphology of the inflorescence of species placed in the section *Eu-agave*, showing its evolution from a much branched panicle to one-sided, flattened, poorly branched umbels. McKelvey and Sax (1933) studied the chromosomes of *Agave americana*, *A. consociata*, *A. filifera*, *A. virginica* and *Furcraea Bedinghausii* and found that in all these there are 30 haploid chromosomes, of which 5 are large and 25 small. They found a similar chromosome complement in *Yucca*, *Hesperoyucca*, *Clistoyucca*, *Hesperaloe* and *Samuela* of the Liliaceae. This resemblance according to the authors points towards a common origin of these genera. Whitaker (1934) found 10 large and 50 small diploid chromosomes in *Furcraea gigantea*, *F. Beding-*

hausii and *F. Selloa* and 36 somatic chromosomes in *Doryanthes Palmeri*. He observed chromosome numbers also in some other monocotyledons and discussed their bearing on the classification of the Liliifloræ. Wunderlich (1936) found mature pollen grains 2-celled in a species of *Agave*.

Doughty (1936) investigated the cytology of seven species of *Agave*. He found 10 long + 50 short somatic chromosomes in *A. angustifolia* and *A. Lespinassei*, 15 long + 75 short chromosomes in *A. cantala*, 20 long + ca. 90 short in *A. Zapupe*, 24 long + ca. 116 short in *A. fourcroydes*, and 24 long + ca. 114 short in *A. Sisalana*. Meiosis was studied in five species. In *A. amaniensis* and *A. angustifolia* the meiotic divisions proceed almost normally. Only rarely chromatid bridges are seen at I anaphase. The pollen is nearly normal. In *A. cantala*, trivalents, bivalents and univalents are observed during the I diakinesis and metaphase. At I anaphase many abnormalities are seen, like lagging univalents and chromatid bridges. There is a large amount of bad pollen, and the seemingly good pollen shows very poor germination. In *A. Sisalana* and *A. fourcroydes*, quinquevalents, quadrivalents, trivalents and univalents are observed during the I diakinesis and metaphase. I anaphase shows many irregularities as observed in *A. cantala*, but the percentage of bad pollen is not so high. The author infers 30 to be the basic number for the genus *Agave* and concludes that *A. cantala* (3x), *A. Sisalana* (5x) and *A. fourcroydes* (5x) are autopolyploids.

Vignoli (1936) investigated the cytology of about 20 species of *Agave* belonging to the sections *Littaea* and *Eu-Agave*. The meiotic divisions in the pollen-mother cells were seen to proceed nearly in a regular manner in *A. Rovelliana*, *A. Bouchei*, *A. Sartorii*, *A. Gilbeyi* and *A. Salmiana* var. *angustifolia*. Only a small number of mother cells were found to degenerate. The divisions were very irregular in *A. Haseloffii*, *A. Warelliana*, *A. micracantha*, *A. Sisalana*, *A. Candelabrum* and *A. Zapupe*, which propagate by bulbils. Following haploid chromosome numbers were reported as new records*: *A. Rovelliana* 25, *A. Bouchei*, *A. Sartorii*, *A. micracantha* (and *A. Haseloffii*?) 30, *A. Salmiana* var. *angustifolia* (and *A. ferox*?) 60, *A. Gilbeyi* 90, which with the exception of 25 form a polyploid series with the basic number 15. The species with many anomalies in the meiotic divisions are apomictic and reproduce by bulbils. *A. Salmiana* is partly apomictic and forms both seeds and bulbils. A certain inclination towards apomixis, however, is shown by all *Agave* species. This along with the great variation in the species is due to more or less hybrid origin of most of the living species.

The writers (Joshi and Pantulu, 1939) have recently published a short note on the anatomy of the ovary of *Polianthes tuberosa*. Chakraverty (1939) has recorded in the bulbs of the same species the

* Only two abstracts of Vignoli's paper in *Bot. Zbl.* have been seen. In these the chromosome numbers of those species only which have been recorded for the first time are cited.

occurrence of anomalous secondary growth of the same type as found in the aerial stems of *Dracaena* and *Yucca*.

Schnarf (1931) has summarized the important embryological features of the tribe Agavoideæ, including the Poliantheæ, as follows: Tapetum of the secretion type; division of the pollen-mother cells simultaneous in *Doryanthes excelsa*, successive in *Polianthes tuberosa* and *Agave* species; ovules throughout bitegmic; a parietal cell is cut off in *Agave*, *Furcraea* and *Doryanthes*; development of embryo-sac follows the Normal-type; endosperm nuclear in *Furcraea cubensis*, of the Helobiales-type in *Doryanthes excelsa*.

The chromosome numbers reported so far in the tribe Agavoideæ are tabulated below:—

*Chromosome Numbers in the Agavoideæ**

Species		N	2 N	Author
† <i>Agave virginica</i>	..	12	..	Schaffner (1909)
† <i>A. virginica</i>	..	12	24	Muller (1912)
‡ <i>A. americana</i>	20 L. + many S	„
† <i>A. rigida</i>	..	7	..	Catalano (1929)
† <i>A. Sisalana</i>	..	7 (L & S)	..	„ (1930)
<i>A. filifera</i>	..	30 (5 L + 25 S)	..	McKelvey and Sax (1933)
<i>A. americana</i>	..	„	..	„
<i>A. consociata</i>	..	„	..	„
<i>A. virginica</i>	..	„	..	„
<i>A. amaniensis</i>	..	30	60 (10 L + 50 S)	Doughty (1936)
<i>A. angustifolia</i>	..	„	„	„
<i>A. Lespinassei</i>	..	„	„	„
<i>A. cantala</i>	..	90/2	90 (15 L + 75 S)	„
<i>A. Zapupe</i>	ca. 110 (20 L + ca. 90 S)	„
<i>A. fourcroydes</i>	..	ca. 140/2	ca. 140 (24 L + ca. 116 S)	„

* In this list L stands for large chromosomes and S for small chromosomes.

† These numbers are obviously incorrect.

‡ Probably some other species was studied.

Chromosome Numbers in the Agavoideae—(Contd.)

Species	N	2 N	Author
<i>A. Sisalana</i> ..	ca. 138/2	ca. 138 (24 L + ca. 114 S)	Doughty (1936)
<i>A. Revelliana</i> ..	25	..	Vignoli (1936)
<i>A. Bouchei</i> ..	30	..	"
<i>A. Sartorii</i> ..	"	..	"
<i>A. micracantha</i> ..	"	..	"
<i>A. Haseloffii</i> ? ..	"	..	"
<i>A. Salmiana</i> var. <i>angustifolia</i> ..	60	..	"
<i>A. ferox</i> ? ..	"	..	"
<i>A. Gilbeyi</i> ..	90	..	"
§ <i>Furcraea altissima</i>	ca. 50 (10 L + 40 S)	Heitz (1926)
§ <i>F. Lindenii</i>	ca. 40 (10 L + 30 S)	"
<i>F. Bedinghausii</i> ..	30 (5 L + 25 S)	..	McKelvey and Sax (1933)
<i>F. Bedinghausii</i>	60 (10 L + 50 S)	Whitaker (1934)
<i>F. gigantea</i>	"	"
<i>F. Selloa</i>	"	"
<i>Beschorneria superba</i>	"	Muller (1912)
<i>B. Yuccoides</i>	60 (12 L + 48 S)	Körperich (1930)
<i>Doryanthes excelsa</i> ..	18-22	..	Newman (1928)
<i>Doryanthes excelsa</i> ..	22	44	" (1929)
<i>D. Palmerii</i>	36 (slight size differences)	Whitaker (1934)
<i>Polianthes tuberosa</i> ..	30 (5 L + 25 S)	..	"

§ The counts of small chromosomes appear to be wrong. It is probable that in both species there are 50 small chromosomes in addition to 10 large.

MATERIAL AND METHODS

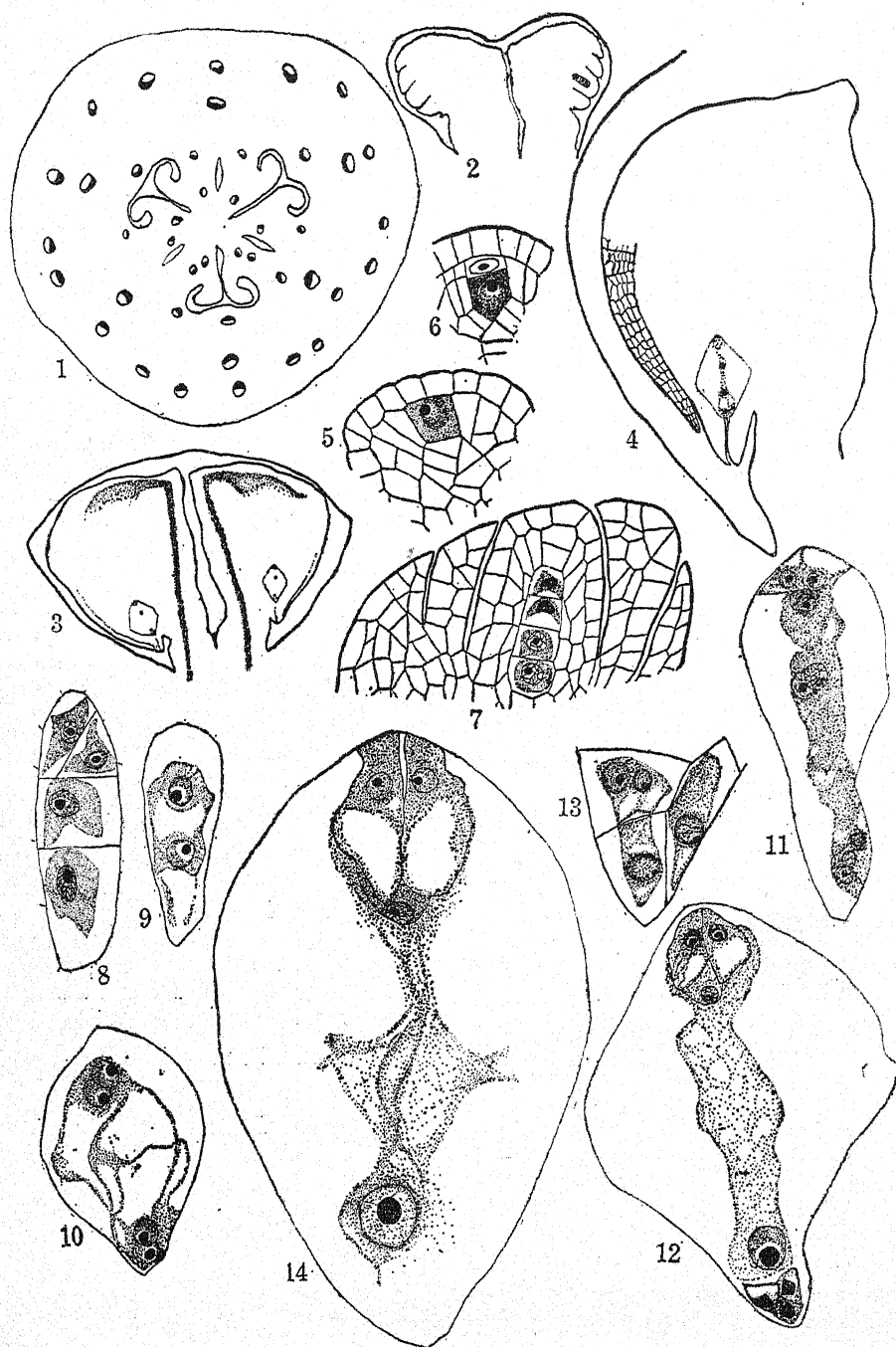
The material of *Polianthes tuberosa* used for the present investigation was entirely collected from the Benares Hindu University gardens and from plants bearing only single flowers. It was fixed during the rainy seasons (July–September) of 1937 and 1938. During this period the plants grow vigorously and flower profusely.

For anatomical and embryological work flowers were fixed in Navashin's fluid and Formalin-acetic-alcohol on sunny days during the hours 10 A.M. to 1 P.M. An exhaust pump was used when fixing material in Navashin's fluid. The washing, dehydration and embedding in paraffin were carried out according to the customary methods. Sections were cut 6–10 μ thick according to the age of the flower and stage desired. Heidenhain's Iron-alum Hæmatoxylin was mostly used for staining, but Delafield's Hæmatoxylin was also used and found more suitable for studying the early stages of anther development.

For cytological work the material was fixed in Flemming's (medium), Benda's and Navashin's fixatives. The hours between 9–30 and 11 A.M. on sunny days were found suitable for the purpose. Before fixing the stage of development of a flower was determined by making an aceto-carmin smear from one of the anthers. In the case of floral material only anthers were fixed. Perianth-leaves and ovaries were separated and excluded. Pretreatment with Carnoy's fluid was tried, but did not yield good results. An exhaust pump, therefore, was used to cause the immediate sinking of the anthers in the fixative. Of the three fixatives used, Benda's and Navashin's gave more satisfactory results, but as many chromosomes in this species are of a small size, Navashin's fixative was preferred to Benda's on account of its high acetic acid content and was more commonly employed. The material was embedded in paraffin according to the directions given by La Cour (1937). Sections were cut 10–12 μ thick and stained with Heidenhain's Iron-alum Hæmatoxylin and Newton's Iodine Gentian Violet (Crystal Violet was used instead of Gentian Violet). To ensure proper staining a solution of 1 per cent. chromic acid was used as a mordant after Crystal Violet. Smears of pollen-mother cells were fixed in Navashin's fixative for one to two hours and stained with Iodine Crystal Violet.

DEVELOPMENT AND ANATOMY OF THE FLOWER

Newman (1928) made a detailed study of floral organogeny in *Doryanthes excelsa*. The present observations on *Polianthes tuberosa* entirely agree with those of Newman. As his account is profusely illustrated, no figures of floral development are given in this paper. The different whorls arise in strict acropetal succession, the order of differentiation being outer whorl of perianth, inner whorl of perianth, outer whorl of stamens, inner whorl of stamens and the carpels. The members of different whorls arise as separate primordia, but soon the processes of adhesion and cohesion begin. The primordia of stamens and the perianth leaves just to their outside unite, and



Figs. 1-14.—*Polianthes tuberosa*. Fig. 1. Transverse section of an ovary showing the arrangement of the vascular bundles. Figs. 2-4. There

this is followed by the union of all the perianth leaves and stamens and the formation of a perianth-stamen tube. Finally the perianth-stamen tube fuses with the carpels and the flower reaches the epigynous stage. The carpels, however, do not lose their individuality completely, so much so that they are not completely united with one another laterally even in the mature condition (Fig. 1).

The most interesting feature of floral anatomy of *Polianthes tuberosa* is that the traces for the various floral parts separate out from the stele of the receptacle below the ovary, and in the wall of the inferior ovary the bundles of the outer and inner whorls of the perianth leaves, stamens and carpels are present quite distinct from one another. This is clear from Fig. 1, which represents a transverse section of the ovary about its middle. There are seen on the outside 18 bundles for the six perianth leaves, each perianth leaf being supplied by three (one dorsal bundle and two lateral bundles). Next there are six stamen bundles, one for each stamen, just to the inside of the six midrib bundles of the perianth leaves. Finally we see in the middle of the transverse section, the vascular supply of the three carpels, consisting in each case of a dorsal bundle, two median lateral bundles at the sides of the carpels and two ventral bundles. The significance of this feature is discussed later.

DEVELOPMENT AND STRUCTURE OF THE OVULE AND EMBRYO-SAC

The ovules in *Polianthes tuberosa* are anatropous, bitegmie and borne in two rows along the margins of each carpel (Figs. 1-4). Newman (1928) says that the ovules in *Doryanthes excelsa* arise from the abaxial side of the carpels and are not exactly marginal. Such origin of the ovules has not been observed in the present material. The nucellus is well developed. Each integument is mostly 2-5 cells thick (Fig. 4). The micropyle is formed by the inner integument only. The vascular supply of the ovule is limited to a single bundle which traverses the whole length of the raphe unbranched and ends blindly in the chalaza (Fig. 3).

The development of the ovules in *Polianthes* agrees exactly with that of *Cimicifuga* described recently by Earle (1938). The ovules after their differentiation from the carpellary margins (Fig. 1) grow straight till they meet the opposing dorsal wall of the carpel (Fig. 2) and then bend outwards and away from one another.

stages in the development of the ovule. Figs. 5-14. Various stages in the development of the embryo-sac. Fig. 5. Primary archesporium. Fig. 6. Formation of the primary wall cell. Fig. 7. A linear tetrad of megaspores. Fig. 8. A T-shaped tetrad of megaspores. Fig. 9. 2-nucleate embryo-sac. Fig. 10. 4-nucleate embryo-sac. Fig. 11. A young 8-nucleate embryo-sac. Fig. 12. A mature 7-nucleate embryo-sac showing the secondary nucleus at the chalazal end. Fig. 13. Antipodals from another embryo-sac showing one 2-nucleate antipodal. Fig. 14. Embryo-sac from a much enlarged ovary after all the other floral parts had withered away. Figs. 1-3, $\times 36$; Fig. 4, $\times 72$; Fig. 8, $\times 720$; the rest, $\times 360$.

Ultimately growing according to the space available, they assume an anatropous form (Figs. 3 and 4). Similar development of the ovules is seen in many other Liliifloræ and other flowering plants like Dilleniaceæ, Magnoliaceæ, Aponogetonaceæ, etc. The significance of this type of development in the origin of the anatropous ovule has been discussed by one of us elsewhere (Joshi, 1935).

There is a single hypodermal archesporial cell (Fig. 5), which differentiates along with the inner integument. It divides periclinally to form a primary wall cell and the megaspore-mother cell (Fig. 6). The primary wall cell divides first anticlinally and then periclinally to form four cells arranged in two layers. These divisions are completed by the time of tetrad formation. In *Doryanthes* (Newman, 1928), the parietal tissue is more extensive and consists of about seven layers at this stage. The megaspore-mother cell undergoes the two meiotic divisions in the normal manner to form a linear (Fig. 7) or occasionally a T-shaped (Fig. 8) tetrad. The chalazal megaspore develops into the embryo-sac according to the Normal-type (Figs. 9-12). The three micropylar megaspores degenerate early. Similar development of the embryo-sac has been observed in other Agavoideæ investigated so far, but according to Nevins (1927) in *Furcraea Andina* the micropylar megaspore functions, while the three chalazal megaspores degenerate.

The 8-nucleate embryo-sac is at first nearly cylindrical (Fig. 11), but it soon begins to enlarge in the middle and assumes a broadly spindle-shaped form (Fig. 12). The egg is flask-shaped and quite normal in structure. In the earlier stages of development the synergids show vacuolation near the micropylar end also (Fig. 11), but these vacuoles ultimately disappear and the synergids have only a large vacuole towards their chalazal end and the nucleus in the micropylar half. In very old embryo-sacs synergids commonly show small hooks (Fig. 14). Only unusual feature noticed is that very rarely an antipodal cell may be 2-nucleate (Fig. 13). Newman (1929) also observed in *Doryanthes* a tendency for division in the antipodals. The polar nuclei meet near the centre of the embryo-sac (Fig. 11), but after their fusion—It has not been possible to ascertain whether before or after fertilisation—they move towards the chalazal end of the embryo-sac (Figs. 12 and 14). In *Doryanthes excelsa*, Newman (1929) has shown that the development of the endosperm corresponds to the *Helobiales*-type. The development of the endosperm in *Polianthes* could not be worked out, but the movement of the secondary nucleus towards the chalazal end of the embryo-sac in this case may be taken as partial evidence for the occurrence of this type of endosperm in this genus as well.

Material was sectioned for studying the development of the endosperm and embryo. For this purpose ovaries were taken from flowers which had bloomed many days ago and all the other parts had completely withered away. The ovaries had increased many times their size at the flowering stage and had to all outward appearance formed fruits. The sections of these ovaries, however,

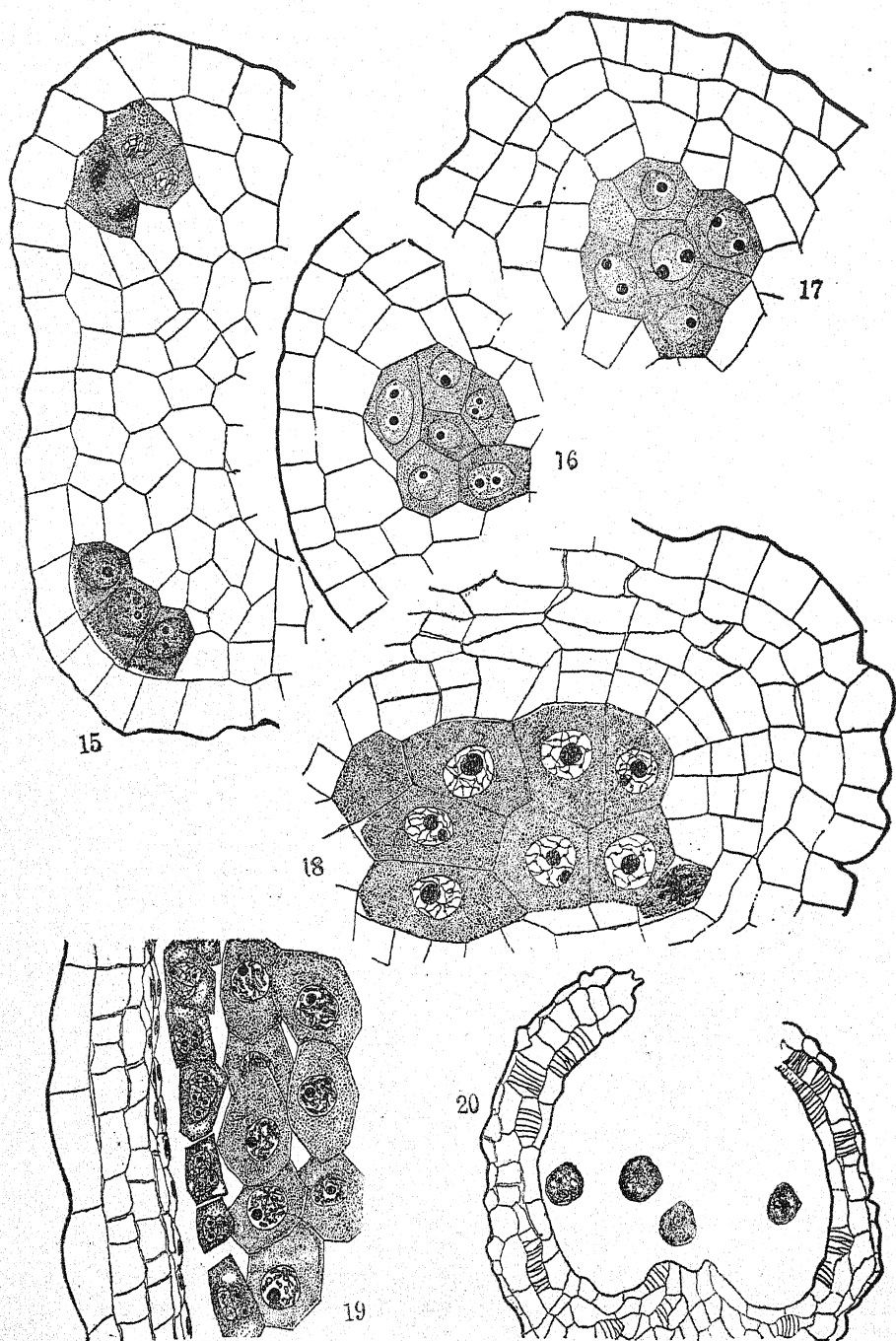
revealed only ovules with very much enlarged embryo-sacs (Fig. 14). There was yet no initiation either of endosperm or embryo development. Due to lack of more advanced material the investigation on this point could not be pursued further.

DEVELOPMENT OF THE ANTHER

Newman (1928) says that in *Doryanthes excelsa* there is no clearly marked archesporium, either hypodermal or more deeply situated, but the sporogenous function appears to settle gradually on a small group of cells situated about three or four layers below the epidermis and similarly the primary parietal function might be regarded as settling gradually on the externally adjoining layers. The tapetum according to him is of sporogenous origin. As both these features are very unusual for angiosperms, an attempt has been made to follow the development of the anther in detail in *Polianthes tuberosa* in order to see how far Newman's account is correct.

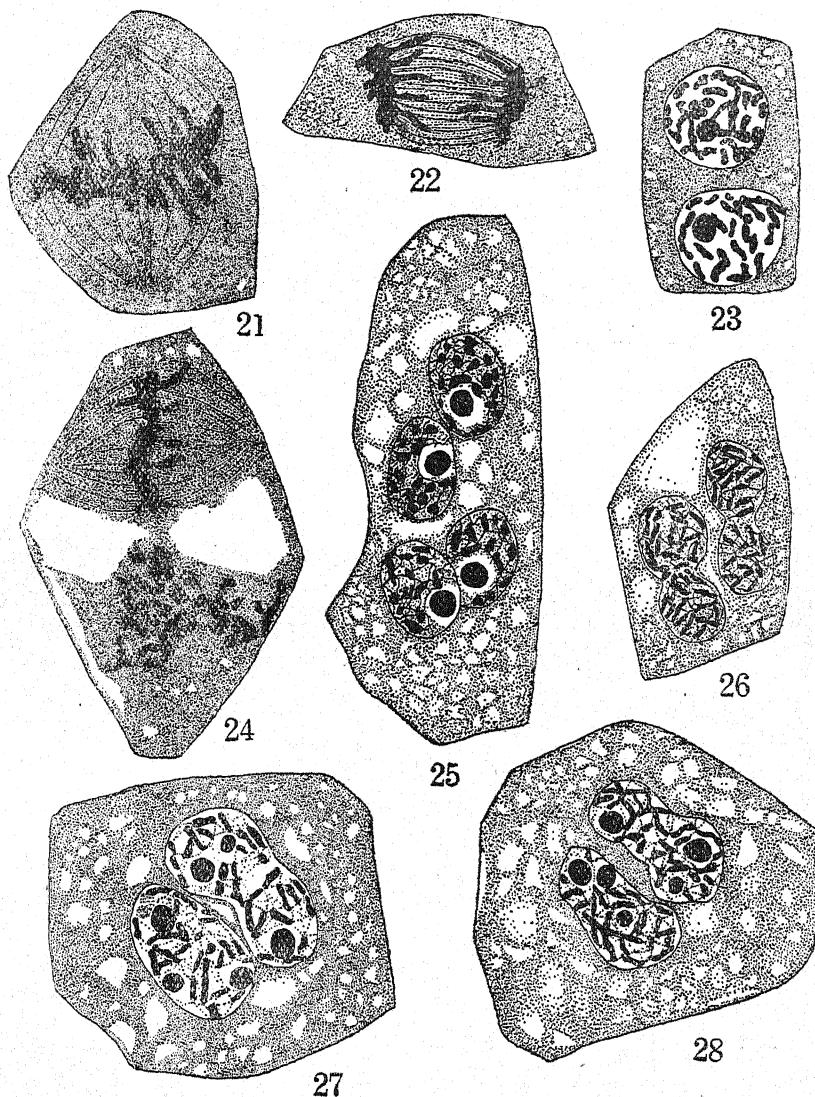
A rough study of anther development in *Polianthes tuberosa* appeared to support Newman's conclusions, but a more careful study, particularly of sections stained with Delafield's Hæmatoxylin, enabled the writers to definitely locate sub-hypodermal archesporium. Two to three cells just below the epidermis in each lobe in a transverse section of the anther were found to increase in size, stain deeper than the rest and show a higher nucleus to cell ratio (Fig. 15). They become still more clear as they divide to form parietal cells towards the outside and primary sporogenous cells towards the inside. The primary sporogenous cells after their differentiation divide very quickly to form a cylindrical, in a transverse section rounded, mass of sporogenous cells (Fig. 16-18). This perhaps misleads one to think that the primary archesporium is not hypodermal in origin. The primary parietal cells divide to form five layers of wall cells (Fig. 19). The innermost wall layer develops into the tapetum, the two layers just outside the tapetal layer degenerate early, while the two outermost of these layers persist and form the fibrous endothecium (Fig. 20). Usually among angiosperms the fibrous endothecium is only one-layered. In *Doryanthes excelsa* according to Newman (1928) the fibrous endothecium is still more extensive. The same feature has also been observed by us in *Gloriosa superba*, one of the Liliaceæ.

The tapetum in *Polianthes tuberosa* is of the secretion type and there is no development of periplasmodium. The tapetal cells are at first uni-nucleate. They always stain more deeply than other cells of the anther and commonly develop large vacuoles. As the pollen-mother cells undergo the two meiotic divisions, the nucleus of the tapetal cells divides mitotically (Figs. 21 and 22) and they become bi-nucleate (Fig. 23). The daughter nuclei undergo one more division (Fig. 24) and thus the tapetal cells become quadri-nucleate (Fig. 25). After this the nuclei of the tapetal cells begin to fuse in pairs and the mature tapetal cells once again become



Figs. 15-20.—*Polianthes tuberosa*. Various stages in the development of anther. Fig. 19 shows a longitudinal section. The rest represent transverse sections. Figs. 15-18, $\times 720$; Fig. 19, $\times 360$; Fig. 20, $\times 80$.

bi-nucleate (Figs. 26-28). Each nucleus is now tetraploid and has got generally four nucleoli. Occasionally three nuclei fuse together forming a hexaploid nucleus and one diploid nucleus remains



Figs. 21-28.—*Polianthes tuberosa*. Various stages in the development of tapetal cells. Fig. 21. First division of the nucleus of a tapetal cell showing metaphase. Fig. 22. Telophase. Fig. 23. A 2-nucleate tapetal cell. Fig. 24. The two nuclei dividing (metaphase). Fig. 25. A 4-nucleate tapetal cell. Figs. 26-28. Various stages in the fusion of the tapetal nuclei. $\times 900$.

separate. Similar mitotic division of the parent nucleus and fusion of the daughter nuclei in the tapetal cells has been observed by Winkler (1906) in *Wickstroemia indica*, Tahara (1905) in *Morus*, Smith (1933) in *Galtonia candicans*, Cooper (1933) in *Podophyllum peltatum*, Bhargava (1936) in *Chenopodium album*, and Raghavan (1938) in *Gynandropsis pentaphylla*. Further examples of the same phenomenon are cited by Bonnet (1912), and Mascère and Thomas (1930). Among plants being studied in this department (unpublished observations) this has been seen in *Polemonium coeruleum* and *Phytolacca* sp. There is no doubt that it is a widespread feature of the tapetal cells lining the pollen-sacs of angiosperms. In many plants the nuclei of the tapetal cells are commonly seen to become multi-nucleolate in later stages. Division of the original nucleus and fusion of the daughter nuclei is the probable explanation of this feature in most cases.

THE MEIOTIC DIVISIONS IN POLLEN-MOTHER CELLS

Resting stage and nucleolus—The pollen-mother cells at the beginning of meiotic divisions are closely packed and of polygonal outline in section. They are densely filled with granular cytoplasm. The resting nucleus of each pollen-mother cell is almost spherical. It shows generally two nucleoli, but occasionally there is only one nucleolus (Figs. 29 and 30). When there is only one nucleolus, it is bigger than either of the nucleoli of a 2-nucleolate nucleus. When there are two nucleoli in a nucleus, one is generally smaller than the other. The same condition has been recorded by Fikry (1930) in *Rumex scutatus*. Bhatia (1938) observes that in wheat of the four nucleoli in a tetraploid race two are bigger and two smaller. Whereas of the six nucleoli in the hexaploid race four are bigger and two smaller. No clear zone around the nucleoli, as observed by Latter (1926) in *Lathyrus odoratus*, was observed in the present material. The writers agree with Majumdar and Datta (1935) and Bhatia (1938) that the appearance of such a zone is an artefact. Similar opinion is held by Fikry (1930), who says, "there is a distinct relationship between good fixation and smallness of the zone".

Many investigators, e.g., Wager (1904) in *Phaseolus*, Fikry (1930) in *Rumex scutatus*, Koshy (1934) in *Allium*, Sheffield (1927) in *Oenothera* and Bhatia (1938) in wheat, have recorded the presence of vacuoles in the nucleoli. Similar vacuolation has been observed in the sectioned material in *Polianthes*, but not in all stages. The nucleoli of the resting nuclei generally do not show vacuolation. The vacuoles are very clear and large during the leptotene and zygotene stages and they decrease in size and tend to disappear during the diplotene and diakinesis stages, but in best fixed material the vacuoles are not prominent. In smear preparations of pollen-mother cells fixed in Navashin and stained with Newton's Iodine Gentician Violet no vacuoles were seen in the nucleoli. The reality of vacuoles in the nucleoli has been a subject for discussion in recent years. Gates and Latter (1927) believe that the vacuolation of the

nucleolus is a normal feature and is caused by loss of nucleolar material and its utilisation in chromatin formation during the prophase. Fikry (1930) disregards this view and regards it as an artefact, and the same view has been expressed by McClintock (1934). Zirkle (1928) found that in *Zea Mays* 2 per cent. acetic acid fixes the nucleoli as large vacuolate bodies, while a mixture of 4 per cent. formalin and 2 per cent. acetic acid fixes the nucleoli as densely staining bodies. The same author (Zirkle, 1931), however, observed that in the living cambial cells of *Pinus* the nucleoli contain small droplets of a less refractive substance and consequently appear vacuolate. Dermen (1933) observed vacuolation of the nucleoli both in living as well as in fixed material and considers it to be a normal phenomenon. Bhatia (1938) agreeing with Dermen's views says that vacuolation is a normal feature of the nucleoli in plants and their absence in some of the nucleoli of the resting stages in wheat only suggests that the flow of nucleolar material from the nucleoli has not yet started. From the present study of *Polianthes tuberosa* it appears that the vacuolation of the nucleolus is partly an artefact and partly a result of some internal changes in the constitution of the nucleolus. That it is an artefact is shown by the absence of vacuoles in smear preparations. That changes in the constitution of the nucleolus also take place is shown by the fact that in microtomed material vacuoles are absent from the resting nuclei, they are large and very prominent during the early prophase stages and decrease in size and tend to disappear during the diplotene and diakinesis stages. These changes may be purely chemical in nature and at present we know nothing about them. They may be associated with the passing out of some nucleolar matter, as believed by Gates and Latter (1927), which is necessary for the development of the chromosomes. On the other hand, it is quite possible that the vacuolation is the result of purely physical changes. In *Polianthes* on the onset of prophase the nucleoli undergo considerable increase in size. This will tend to reduce their density and make them liable to develop vacuoles under the action of certain fixatives. Later the nucleoli may be able to stabilise themselves with the changed conditions. As the prophase stage advances, they may be able to assimilate some further material. Their density may also increase due to decrease in volume, such as has been noticed in rice by Parthasarathy (1938) from the leptotene to the diakinesis. The vacuoles thus gradually disappear. An analogical comparison in this respect may be made with the development of the pollen grains after their liberation from the mother cells. They undergo a sudden increase in size and become highly vacuolate. Later, however, the vacuoles are gradually filled up.

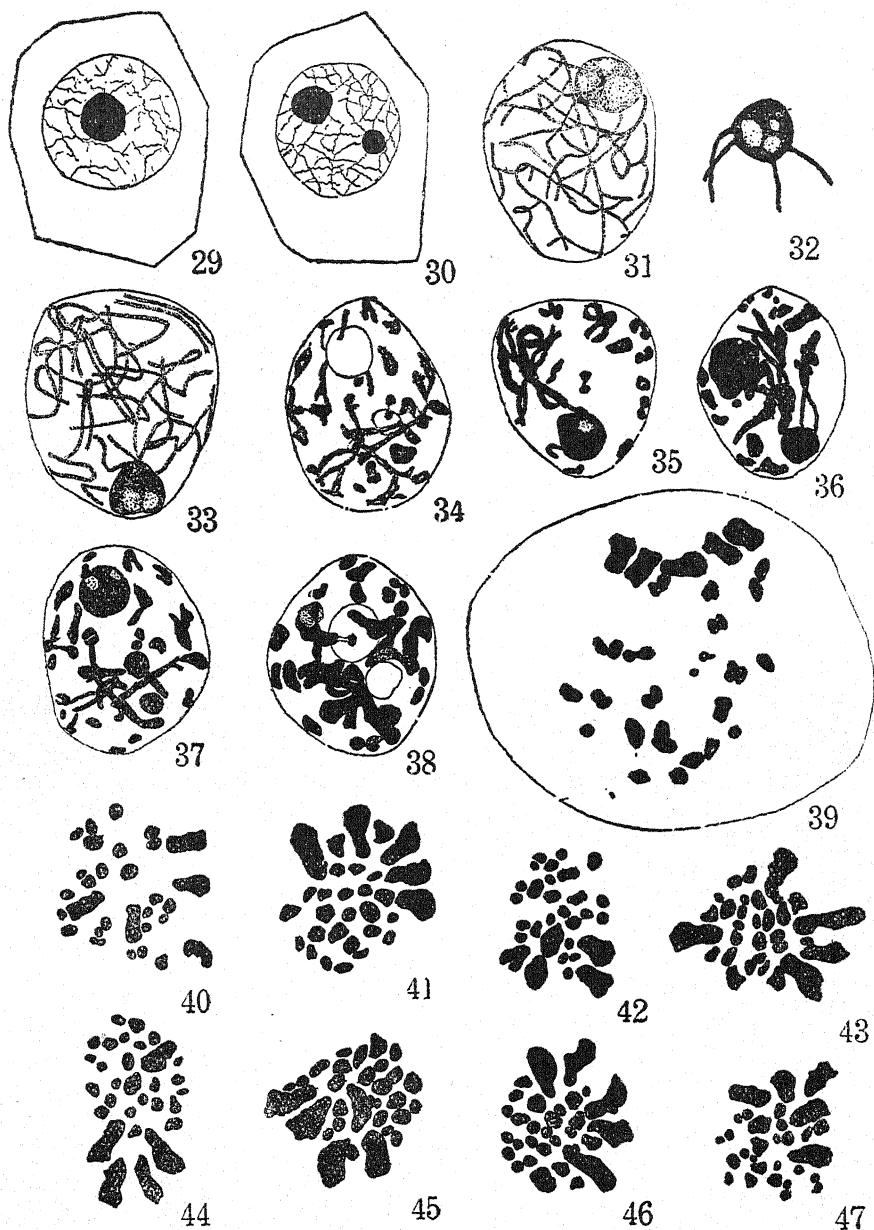
Latter (1926) in *Lathyrus*, Sheffield (1927) in *Oenothera* and Bhatia (1938) in wheat have observed some crystalline bodies in the nucleoli. In the present material no such bodies were observed.

Leptotene.—With the onset of prophase the nuclei of pollen-mother cells increase in size and the nearly invisible chromosomes become clearly visible as the delicate leptotene threads. These

threads are dispersed without any regularity or orientation throughout the nucleus. They can be followed to a considerable length, but their relationships cannot be exactly made out. At this stage only one nucleolus is seen in the nuclei of most of the pollen-mother cells (Fig. 31). This is due to the fusion of the two nucleoli seen at the resting stage. Raghavan (1938) in *Gynandropsis pentaphylla* has also observed that the two nucleoli of the resting stage fuse on the onset of the I meiotic division. Similar fusion of nucleoli has also been observed by Bhatia (1938) in wheat and Parthasarathy (1938) in rice. In a few cases, however, two nucleoli have been observed at this stage and they show the usual size difference.

The question, why these separate nucleoli fuse at this stage, has been discussed by Parthasarathy (1938) for rice. He states that there can be only two possible reasons. First, the nucleoli come together due to attraction between the pairs of nucleolar chromosomes which are ancestrally homologous. He admits that it is not known whether such a secondary attraction exists at this stage, but supports this view by stating that in early leptotene in rice the nucleoli are always near each other, from which it may be inferred that the nucleolar chromosomes are also always close together. Further the occurrence of somatic pairing in rice indicates the proximity of at least some of the homologous chromosomes in prophase. His second suggested explanation, though he himself rejects it, is that the fusion between two nucleoli could take place by slight increase in the size of the nucleoli during the meiotic prophase, bringing them in contact. Both of these explanations do not appear to be applicable to the conditions found in *Polianthes tuberosa*. In this species, whenever there are two nucleoli in a nucleus in the resting or early leptotene stages, these are generally found to be situated quite far apart (Fig. 30). There is thus no evidence that pairs of nucleolar chromosomes show any secondary attraction for each other at this stage. Further during the diplotene and diakinesis stages, as will be described later, when such secondary attraction should really develop, the single nucleolus very often breaks into two once again and the two pairs of nucleolar chromosomes are also seen to fall apart along with this. To suppose that the separate nucleoli meet and fuse during the leptotene stage simply as the result of increase in size, which they do undergo at this stage, is also refuted by the great distance between them. The only possible explanation, therefore, is that the nucleoli themselves attract each other and tend to come together, so that even when they are far apart they move to one point and coalesce. In many nuclei this process of nucleolar fusion is completed from the telophase to the resting stage. In other nuclei, where the nucleoli may remain separate during the resting stage, they come together and fuse during the early prophase.

At leptotene four chromosome threads are seen attached to the nucleolus (Figs. 31 and 32), and this attachment continues during



Figs. 29-47.—*Pollianthes tuberosa*. Various stages of the I meiotic division in pollen-mother cells up to metaphase. Figs. 29 and 30. Two mother cells with resting nuclei. Figs. 31 and 32. Leptotene. In Fig. 32 only the nucleolus with the attached chromosomes is shown. Fig. 33. Zygotene. Figs. 34-37. Diplotene. Fig. 38. Diakinesis. Figs. 39 and 40. Two prometaphase stages. Figs. 41-47. Seven representative metaphase plates. $\times 1500$.

the later prophase stages till the disappearance of the nucleolus at metaphase. Similar attachment of the chromosomes with the nucleolus has been by now observed in many other plants and animals (Gates, 1937).

Diplotene—During zygotene the threads conjugate in a parasyntaptic manner (Fig. 33). There is nothing remarkable to mention about the pachytene stage in *Polianthes*. During diplotene the parallel association of the conjugating chromosomes is disturbed by the appearance of chiasmata. In the large chromosome pairs generally 4 or 5 chiasmata are observed. The larger of the small chromosome pairs generally show two terminal chiasmata, which give the bivalents ring-like appearance. The small chromosome pairs have only one chiasma. It is thus clear that the number of chiasmata per bivalent depends upon the length of the chromosomes. In the large chromosomes the double nature of the homologues becomes visible at this stage.

Most of the large chromosomes (4 pairs) at this stage segregate towards one side and form a close group, while the small chromosomes remain distributed throughout the nucleus (Figs. 34 and 35). The nucleus thus begins to show a kind of polarity. In many nuclei two nucleoli again appear at this stage, one of which is larger than the other (Fig. 36). On prolonged destaining the smaller nucleolus disappears before the larger one. In rare cases three nucleoli are observed, out of which two are small and one large (Fig. 37). When there is only one nucleolus, four chromosomes are found attached to it. When there are two nucleoli, each is attached separately to a pair of chromosomes. The larger of the two nucleoli is found attached to the large chromosome pair which remains a little apart from the other large chromosomes, while the smaller nucleolus is attached to a pair of large chromosomes (Fig. 36), which forms a close group with the three other pairs of large chromosomes. This nucleolus is commonly found enclosed in the clump formed by these chromosomes, and thus is frequently difficult to make out. The larger nucleolus at this stage frequently shows a small protuberance on one side. This area destains more easily than the rest of the nucleolus and the chromosomes are attached to the nucleolus on this protuberance. The pair of chromosomes attached to the large nucleolus at this stage and during the following diakinesis stage (Fig. 38) is seen to be satellited, but no satellites could be observed on the chromosomes attached to the small nucleolus. This may be due to the satellites in this case being separated from the body of the chromosomes by very short filaments. Bhatia (1938) has also observed that in wheat ordinarily the number of nucleoli in a nucleus from the zygotene onwards is only one, but occasionally two nucleoli are seen. He interprets the presence of two nucleoli at this stage as the result of failure to fuse at the early stages. This may be the case in some nuclei in the present material also, for occasionally two nucleoli have been observed at the leptotene and zygotene stages. The number of nuclei showing two nucleoli at the diplotene stage, however, is much larger. It is, therefore,

clear that the higher number in many cases is due to the breaking of the former single nucleolus. This may happen because at this stage the two chromosome pairs attached to it undergo great contraction and may pull in different directions. The development of the third nucleolus is most probably due to a split in the small nucleolus, which may be caused by the moving apart of the ends of the two chromosomes attached to it.

Diakinesis—At diakinesis also the large chromosomes except the one in association with the large nucleolus continue to lie on one side as in the diplotene, but not in such a close group. All the bivalents are arranged around the periphery of the nucleus. At this stage the complete haploid chromosome number can be clearly counted (Fig. 38). The nuclear membrane now is very delicate. The nucleolus continues to stain as deeply as in the previous stages. Neither there is any reduction in its size. This is contrary to what has been noted by Verbrugge (1934) in *Oenothera*, Fikry (1930) in *Rumex scutatus*, Nandi (1937) in rice and Bhatia (1938) in wheat. On the other hand the present observations agree with those of Dermen (1933) on *Callisia* and *Pinus*.

I Metaphase—At the end of diakinesis (Fig. 39) the nucleolus and the nuclear membrane suddenly disappear. The chromosomes spread out in the cell, reach the maximum lengthwise contraction and assume the equatorial position. The polar view now clearly shows the haploid complement of 30 chromosomes as observed by Whitaker (1934), and these show distinct size differences. The whole complement can be easily divided into two groups, large and small; 5 chromosomes belong to the first group and 25 to the second. Out of the 25 small chromosomes about 10 are larger than the other 15. Figs. 41–47 illustrate some representative metaphase plates. These show that out of the five large bivalents, four are always found on one side, while the small bivalents are mostly aggregated on the other side. The fifth large bivalent may be situated close to the other large bivalents (Figs. 41, 46 and 47) or may be found on the side away from them (Figs. 43 and 44). Both the arrangements are nearly equally common. Such an arrangement of bivalents therefore is very similar to what is seen during the diplotene and diakinesis stages. It is usual to explain the arrangement of the bivalents at metaphase according to the laws governing the configurations formed by floating magnets, first formulated by Mayer (1879) and further worked out by Cannon (1923) and Kuwada (1929). It should be interesting to test experimentally how far the arrangements seen in *Polianthes tuberosa*, where the chromosomes are of unequal size, can be explained in accordance with these laws. In heavily destained preparations on the average 3 chiasmata were counted per large bivalent. Thus as usual there is considerable decrease in chiasma frequency from the diplotene to the metaphase stage.

Even from the beginning of prometaphase (Figs. 39 and 40) some bivalents are found to form groups of two or more. This

secondary pairing of the chromosomes is seen in many metaphase plates, though not in every one (Figs. 40-47). This quite agrees with the views of Catcheside (1937), who concludes from statistical analysis that secondary pairing is dependent upon the relative positions of bivalents at diakinesis, and the bivalents which happen to lie adjacent at diakinesis and which are capable of secondary pairing are so paired at metaphase. So there is as much chance of maximum association as there is for no association or random distribution. Further the secondary pairing is observed only in relation to small chromosomes, as reported by Darlington (1928). The exact amount of maximum secondary pairing was found difficult to determine. Many counts made did not lead to any conclusive result. This may be due to the large number of chromosomes and their close distribution in a small space. Many may be coming together merely on account of the similarity of their size. The chief reason, however, most probably is the presence of large chromosomes among the small ones. By coming in between the smaller chromosomes they obstruct secondary pairing of some of the smaller chromosomes also and thus cause irregularities in the expression of secondary pairing. Besides groups of two, groups of three bivalents are also common, and occasionally groups of four bivalents are also observed. Often the bivalents are found arranged in radial rows, a feature which is seen in high polyploids. In addition to secondary pairing between small bivalents, one small bivalent is commonly found in association with a large one.

I Anaphase to II Telophase—The I anaphase proceeds in the normal manner. Side views (Figs. 48 and 49) show that the homologous chromosomes are equally distributed on both sides of the equatorial plane. The small chromosomes reach the poles before the larger ones, but no lagging of any bivalents is observed. The secondary association seen at metaphase is maintained to some extent during anaphase also.

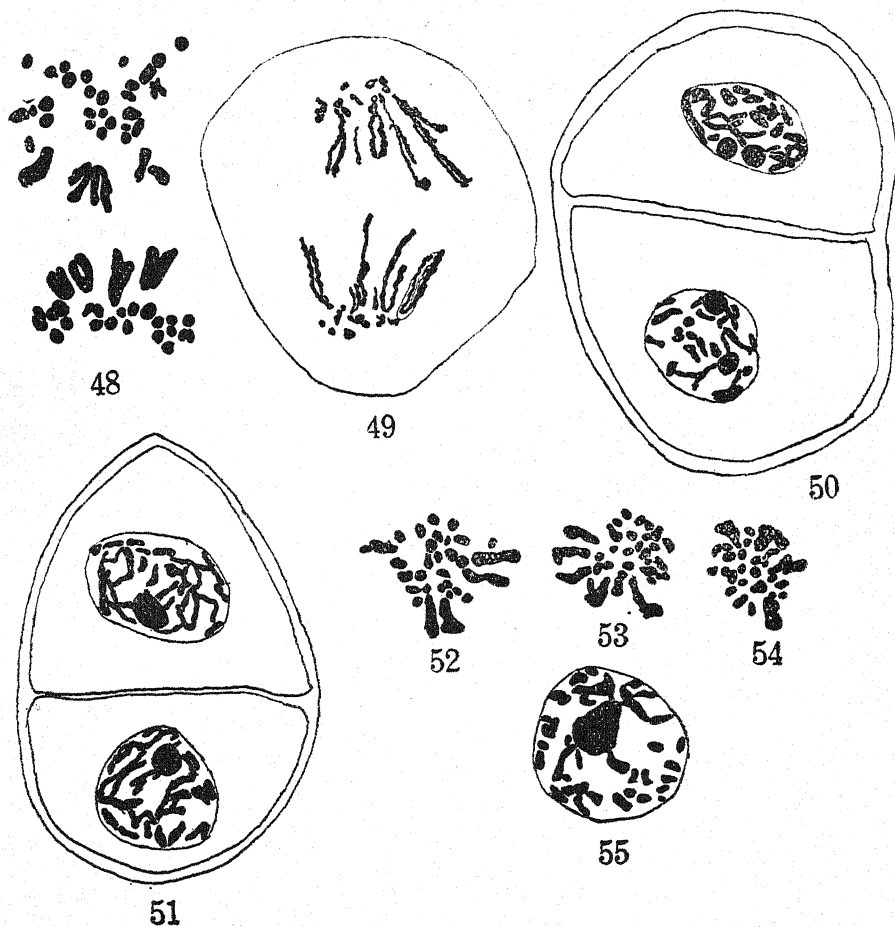
After the chromosomes have reached the poles, each group organizes into a telophase nucleus (Figs. 50 and 51). The nucleoli make their appearance. Generally two nucleoli are formed (Fig. 50), out of which one is somewhat larger than the other. Later these fuse to form one nucleolus (Fig. 51). After the I meiotic division, a cell wall is laid down, which divides the pollen-mother cells into two cells.

The II meiotic division also proceeds in the normal manner. At metaphase II secondary association is again observed (Figs. 52-54). During the II telophase the distribution of the chromosomes resembles that found during interkinesis.

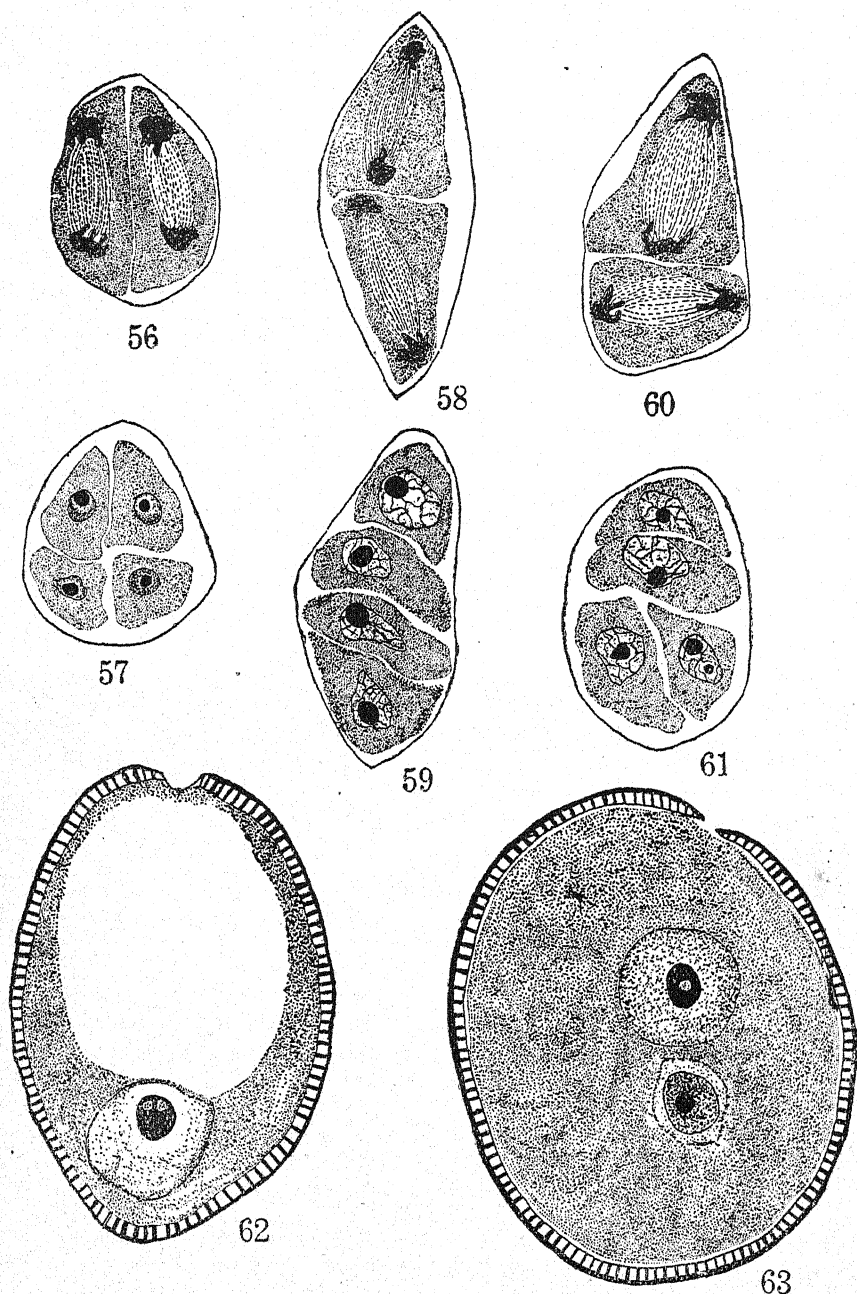
CYTOKINESIS AND MATURE POLLEN

The formation of pollen grains in *Polianthes tuberosa* corresponds to the *Successive*-type. In this respect this genus differs from *Doryanthes*, in which the pollen grains are formed according to the *Simultaneous*-type, as in most dicotyledons. The arrangement of

the pollen grains in the tetrads in *Polianthes* is considerably variable. The tetrads may be bilateral, linear or T-shaped. This depends largely on the form of the pollen-mother cells. If the pollen-mother cells are nearly spherical, during the second meiotic division the two spindles are parallel and the resulting tetrad is bilateral (Figs. 56 and 57). If the pollen-mother cells are comparatively much elongated, the spindles during the II meiotic division are in the same line and the resulting tetrad is linear (Figs. 58 and 59). If the pollen-mother cells are of an intermediate shape and rather broad at one end and narrow at the other, the two spindles during the II meiotic division are at right angles to one another and the resulting tetrad



Figs. 48-55.—*Polianthes tuberosa*. Further stages of meiosis in pollen-mother cells after the I metaphase. Figs. 48 and 49. I anaphase. Figs. 50 and 51. Interkinesis. Figs. 52-54. II metaphase. Fig. 55. A root tip cell in the prophase stage showing four chromosomes attached to the nucleolus. $\times 1,500$.



Figs. 56-63.—*Polianthes tuberosa*. Formation of pollen tetrads. Figs. 56 and 57 show the formation of a bilateral tetrad, Figs. 58 and 59 of a linear tetrad, and Figs. 60 and 61 of a T-shaped tetrad. Fig. 62. 1-nucleate pollen grain. Fig. 63. 2-celled mature pollen grain. $\times 1,500$.

is T-shaped (Figs. 60 and 61). Similar variation in the form of the pollen tetrads has been observed in a number of other plants. Schaffner (1897) and Wodehouse (1935) found in *Typha* the tetrads not only indiscriminately tetrahedral and bilateral, but also frequently linear. The same condition has been observed by Frye (1901) and Gager (1902) in *Asclepias*, Narasimhamurthy (1935) in *Ottelia*, Johri (1936) in *Butomopsis* and Sax and Husted (1936) in *Periploca*.

The pollen grains develop the usual exine and intine, leaving one germinal furrow (Figs. 62 and 63). A large vacuole is seen in the 1-nucleate pollen grains, as is common among flowering plants, so that the nucleus is pushed towards one side (Fig. 62). This side is opposite to the germinal furrow. Here the nucleus divides forming a large tube nucleus and a small generative nucleus, which is later organised into a generative cell. After this division the vacuole is gradually filled up and the pollen grains are shed in this 2-celled condition (Fig. 63). This agrees with the observations of Newman (1929) and Wunderlich (1936) on *Doryanthes* and *Agave*. The pollen grains do not show any degenerations and are quite normal.

DISCUSSION

Origin of the Inferior Ovary—There are two main views about the origin of the inferior ovary or perigynous and epigynous conditions in the flowering plants. According to the first view the calyx-tube is of receptacular origin and is the result of cup-like growth of the receptacle around the gynæcium or the invagination of the apex of the floral axis. This view is largely based on ontogenetic studies and is more commonly given in the text-books. According to the second view the calyx-tube is entirely of appendicular origin and is the result of fusion of the bases of sepals, petals and stamens and the inferior ovary represents adnation in its extreme form. This view is largely based on anatomical evidence and is supported among others by Eames (1931), Eames and McDaniels (1925), Jackson (1933, 1934), McDaniels (1937), Moore (1936) and Kausik (1940). Saunders (1925) also on the basis of the leaf-skin theory of the nature of the shoot refutes the receptacular theory and supports the appendicular origin of the inferior ovary.

Newman (1928) from his study of the floral ontogeny of *Doryanthes excelsa* says that the evidence does not fit with the receptacular origin of the inferior ovary. Even from ontogenetic evidence he supports the appendicular theory. In *Polianthes tuberosa* the development of the flower is, as said before, quite similar to that of *Doryanthes*. In addition to this the evidence from anatomy is even more decisively in favour of the appendicular theory. We find even in the wall of the inferior ovary the traces of all the six perianth leaves, six stamens and three carpels quite separate from one another. There is thus no doubt here that the inferior ovary has resulted in this case from the fusion of the bases of the perianth-leaves, stamens and carpels.

From ontogenetic study we find that during development of flower the process of adnation first affects perianth leaves and stamens, and then the carpels. The ultimately epigynous flower, therefore, passes through a perigynous state. This shows that the ovary has developed from perigyny.

Cytogenetical Constitution of Polianthes tuberosa—In recent years important criteria have been established for determining the cytogenetical constitution of a species and elucidating its past history. The first one is the number of nucleoli formed during telophase and the number of chromosomes concerned in their organization (Gates, 1939, and 1939). It appears that in the nucleus of a diploid plant or animal species there is one pair of chromosomes usually with satellite or secondary constrictions which is especially concerned in the organization of the nucleolus. During the prophase of mitosis or meiosis divisions this pair is attached to the nucleolus. At telophase each of these chromosomes produces a nucleolus. In a chromosome with a secondary constriction the nucleolus arises at the secondary constriction. In a satellited chromosome the exact point of origin of the nucleolus appears to be just where the delicate constriction which carries the satellite is attached to the main body of the chromosome. This locus has been called the nucleolar organizing center.

The number of nucleoli formed during telophase, therefore, is usually the same as the number of these nucleolar chromosomes. In a diploid organism two nucleoli will be formed during the mitotic telophase and one during the meiotic telophase. In a polyploid organism the number of these nucleolar chromosomes and telophase nucleoli is correspondingly increased. Thus a triploid will have three, a tetraploid four and hexaploid six chromosomes associated with the nucleolar organization and the same number of nucleoli will be formed during the mitotic telophase, though later this number commonly decreases due to fusion.

The second criterion is the phenomenon of secondary pairing, first observed by Kuwada (1910) in *Oryza sativa* and by Kuwada (1911) in *Dahlia variabilis*, but its relation to the homology of associated bivalents and its independence of meiotic pairing was not recognized only recently through the work of Darlington (1937), Darlington and Moffett (1930) and Lawrence (1931). According to Darlington (1937), secondary pairing appears at the metaphase, may continue during the I anaphase and disappears during the II metaphase. It takes place between bivalents or chromosomes of similar size and shape, and consists in approximation but not in contact. It indicates distant homology between the pairing bivalents and is connected with allopolyploidization. Although doubt has been cast recently on any hypothesis of secondary pairing by Catcheside (1937), Heilborn and Propach (1937), much importance has been attached to the character in their cytogenetical work by Muntzing (1933) on *Polianthes tuberosa*, Moffett (1934) on *Crataegus*, *Mespilus*, *Cotoneaster*, etc., Catcheside (1934) on *Brassica*, Wanscher (1934) on

Empetrum, Matsuura (1935) on *Dicentra*, Alam (1936) on Indian oleiferous Cruciferae, Sakai (1935), Nandi (1936), Parthasarathy (1938) and Ramanujam (1938) on *Oryza*, Raghavan (1938) on Capparidaceae, etc. The theory of secondary pairing if judiciously employed may be regarded well established as a helpful instrument in the determination of the basic number of chromosomes of the species and tracing their past history.

In *Polianthes tuberosa*, there are two pairs of chromosomes attached to the nucleolus or nucleoli during the prophase of the first meiotic division. Two nucleoli are formed at the first meiotic telophase. During the prophase of mitosis in root-tip cells four chromosomes have been found attached to the nucleolus (Fig. 55). This shows that *Polianthes tuberosa* is really a tetraploid form. Further the secondary pairing of bivalents at the I meiotic metaphase reveals not only groups of two, but also commonly groups of three or occasionally even groups of four. The species therefore is not a simple polyploid but a secondary one. The two nucleoli formed during telophase are of unequal size. Also in the resting nuclei or during the prophase, when there are two nucleoli, these differ in their size. The two pairs of chromosomes associated with the formation of the nucleoli are also not exactly similar. They remain mostly away from each other. One of them shows satellites clearly even during the meiotic prophase, but the other was not found to show any satellites even after careful search in the same preparations. Hedayetullah (1933) has found that the Indian variety of rice "Kochivittu", with two large nucleoli of equal size, when crossed with "Nabatat" an Egyptian variety with one large nucleolus, gives hybrids with two unequal nucleoli in the pollen-mother cells. One may guess that the nuclear structure of *Polianthes tuberosa* also originated by crossing in the same manner between two different ancestors with unequal nucleoli. Later the stable condition of chromosomes may have been established by doubling of the chromosome complement of the hybrid through amphidiploidy. Duplication of some of the chromosomes through non-disjunction or some other cause either in the ancestral types or in the hybrid derived from them also appears to have played some part in the evolution of the present number.

The conclusion, therefore, is that *Polianthes tuberosa* is probably a secondarily balanced allotetraploid, whose chromosome complement has been derived from hybridisation between two ancestral parents, followed by amphidiploidy and duplication of some of the chromosomes. It is not possible to fix the exact basic number. As has been mentioned earlier, the phenomenon of secondary pairing is not able to manifest itself fully in this species on account of the presence of some large chromosomes in the complement, and it does not yield either consistent or constant results with regard to maximum association. As the chromosome complement of *Agave* species is exactly similar to that of *Polianthes tuberosa*, the view of Doughty (1936) that 30 is the basic number for that genus appears to be incorrect. The view of Vignoli (1937) that 15 is the basic

number is more correct, but even this number appears to have been derived from a lower basic number. Whitaker (1934) has shown from the comparison of the chromosomes of the Agavoideæ with that of other monocotyledons that this characteristic complement of 5 long and 25 small chromosomes may have been derived from some members of the Helobiales, which is regarded by many botanists as the most primitive order of monocotyledons. Several members of the families Butomaceæ, Hydrocharitaceæ, Najadaceæ and Triuridaceæ show complements with some chromosomes much larger than the rest. We may await detailed cytological study of these families and other monocotyledons allied to the Agavoideæ before trying to determine the exact basic number for the genera of this tribe.

The cause of sterility—Sterility among plants is due to many causes. The present investigation has yielded only negative evidence with regard to the cause of marked sterility in *Polianthes tuberosa*. It has shown that the sterility in this species is not due to any defects or deformities in the formation of the pollen grains or development of the embryo-sac. It will require further experimental work to find out the exact cause.

The form of the tetrad—A very important difference between the processes of microsporogenesis and megasporogenesis among seed plants lies in the form of the tetrad. The tetrads of pollen grains are usually tetrahedral or bilateral, while the tetrad of megaspores is generally linear, though many variations from this rule are known to exist. In many flowering plants the tetrad of megaspores is often T-shaped, a form intermediate between the linear and the bilateral. The process of microsporogenesis in *Polianthes* is interesting in showing great variation in the form of the pollen tetrads. These are almost as commonly linear or T-shaped as bilateral. Further this variation depends entirely on the form of the mother cells. Elongated mother cells give rise to a linear tetrad, the nearly spherical to a bilateral tetrad, and those of an intermediate form to T-shaped tetrad. Among the Archigoniatae the tetrad of spores is either bilateral or tetrahedral. The linear tetrad of megaspores of seed plants, therefore, must have originated at some time from a tetrahedral or bilateral type. The variation in the form of the pollen tetrads in *Polianthes* indicates that this may have been accomplished merely by a change in the form of the mother cell. How that happened is a more difficult question, but it may be guessed that this took place simultaneously with the integumentation of the sporangium (nucellus). It may have resulted merely from mechanical causes, for instance, due to a large number of mother cells developing simultaneously. The form has now become constant due to its better adaptation to the nutritional needs of the embryo-sac, as it tends to directly connect the growing embryo-sac with the vascular supply of the ovule ending in the chalaza.

Origin of the tapetum—In the early literature on the embryology of the angiosperms several examples are found in which the

tapetum is believed to originate from the sporogenous tissue. Recent work has shown in many cases this belief to be based on erroneous observation. Thus Coulter (1898) says that in *Ranunculus* in some cases the tapetum seems to be cut off from the periphery of the sporogenous tissue and in others it is derived from the parietal cells. Similar sporogenous origin for the tapetum is claimed by Swingle (1908) in *Myosurus minimus*. The recent study of *Ranunculus sceleratus* by Singh (1936), however, shows clearly that the conclusions of these authors are incorrect, and the tapetum in the Ranunculaceae is of parietal origin. Rosenberg (1901), Holmgren (1913), etc., claim a sporogenous origin for the tapetum in many Helobiales, but Johri (1935 and 1936) and Sâné (1939) have shown that this is not so at least in the Alismaceae, Butomaceae and Aponogetonaceae.

As Newman (1928) had claimed that there is no clearly marked hypodermal primary archesporium in the anthers of *Doryanthes excelsa* and the tapetum is of sporogenous origin, and his work is illustrated by a large number of figures giving a very close series of developmental stages, special attention was paid during the present investigation to study this aspect of the floral development in *Polianthes tuberosa*. The investigation has definitely shown the hypodermal origin of the primary archesporium and parietal origin of the tapetum, a feature which seems to be common to the vast majority of angiosperms. The observations of Newman thus appear to be doubtful and require re-investigation. It is also clear that in future any claims for a sporogenous origin of the tapetum in flowering plants should be accepted with great caution.

Some taxonomic problems—The bearing of the cytological data on the classification and relationships of the Agavoideae has been previously discussed by Heitz (1926), McKelvey and Sax (1933), Whitaker (1934) and Anderson (1937). The present investigation as far as it goes lends further support to the conclusions of these authors. The tribe Agavoideae of the family Amaryllidaceae as defined in the Pflanzenfamilien (Pax and Hoffman, 1930) includes 7 genera. Chromosome counts have been made in all these except *Prochnyanthes* and *Pseudobravia*. The study of *Agave*, *Furcraea*, *Beschorneria* and *Polianthes* has revealed great uniformity. The haploid complement in every case has been found to consist of 5 large and 25 small chromosomes, or in some species of *Agave* of a multiple of this number with nearly the same proportion between large and small chromosomes. This complement, therefore, is a characteristic feature of these genera. In the genus *Doryanthes*, however, there are only 18 haploid chromosomes and these do not show any such size differences as shown by the other four genera. Hutchinson (1934) in his recent book has split those 7 genera on the basis of floral symmetry and the form of the inflorescence into two tribes, Agaveae (including *Agave*, *Furcraea*, *Beschorneria* and *Doryanthes*) and Poliantheae (including *Polianthes*, *Prochnyanthes* and *Pseudobravia*). According to cytological evidence, if any genus needs separation from the other genera, it is *Doryanthes* and

not *Polianthes* or the related genera *Prochnyanthes* or *Pseudobravoæ*, whose cytology is unknown. This view, as already pointed out by Whitaker (1934), is also supported by evidence from geographical distribution. *Doryanthes* has 3 species confined to Australia. All the other genera are natives of tropical and subtropical America, Mexico being the chief centre of their distribution. Further according to Newman (1928), the pollen grains in *Doryanthes* are formed according to the *Simultaneous*-type. In the other genera studied so far they are formed according to the *Successive*-type (Schnarf, 1931). The recent work of Schnarf and Wunderlich (1939) on the Liliaceæ has shown that the method of pollen formation is distinctive for tribes. Externally these differences between *Doryanthes* and other genera are paralleled in the attachment of the anthers to the filament. In *Agave*, *Furcræa*, *Beschorneria*, *Polianthes*, *Prochnyanthes* and *Pseudobravoæ*, the anthers are dorsi-fixed. In *Doryanthes* alone among the 7 genera of the Agavaceæ, the anthers are basi-fixed. Therefore, if the group has to be divided into two tribes, it should be as follows:—

- I. *Agaveæ*. Anthers dorsi-fixed.—*Agave*, *Furcræa*, *Beschorneria*, *Polianthes*, *Prochnyanthes* and *Pseudobravoæ*.
Distribution : Tropical and sub-tropical America.
- II. *Doryantheæ*. Anthers basi-fixed.—Only genus *Doryanthes*.
Distribution : Australia.

Next we may consider the relationships of *Agave* and allied genera with other Liliifloræ. The investigations of Morinaga *et al.* (1929), Lewitsky (1931), O'Mara (1932), McKelvey and Sax (1933), Whitaker (1934), Sato (1934) and Watkins (1936) have brought out that the chromosomal constitution of the members of the tribe Yuceæ is very similar to that of Agaveæ. In every investigated species of *Hesperalæ*, *Yucca* and *Samuela* also 5 large and 25 small haploid chromosomes have been counted. If we compare the figures of the metaphase plates of *Polianthes* given in this paper with similar figures of *Yucca* species published by Watkins (1936) and other authors, no difference can be made out between the two. Hence, as Anderson (1937) says, it is impossible to believe that such a distinctive chromosome complement could have arisen independently in both the Liliaceæ and Amaryllidaceæ. Further the Yuceæ have the same distribution as the Agaveæ. Any natural system of classification, therefore, must bring the tribes Agaveæ and Yuceæ within the same family. Cytology, therefore, strongly supports Hutchinson's (1934) system, which brings them into a common family Agavaceæ, distinct both from the Liliaceæ and Amaryllidaceæ; but how far the inclusion of Dracæneæ, Phormiææ, Nolineæ and *Doryantheæ* within the same family is justified may be left to future research. The small amount of cytological work that has been done on these tribes gives little guidance for a full discussion of the subject. McKelvey and Sax (1933) have reported 19 *n* chromosomes in *Dracæna arborea*, Whitaker (1934) 19 *n* in *D. fragrans*, McKelvey and Sax (1933) ca. 19 *n* in *Nolina spectabilis*,

Whitaker (1934) 18 *n* in *N. recurvata* and ca. 19 *n* in *Dasyllirion longissimum*. In all cases the chromosomes do not show any marked size differences. As stated before, the chromosomes of *Doryanthes* also show nearly the same number and morphology. It thus seems possible that future work may support the classification of the Agavaceæ into two sub-families,—(I) *Agavoideæ*, including Yuccææ and Agavææ, and (II) *Dracænoideæ*, including Dracæneæ, Phormiææ, Nolineæ and Doryantheæ. These sub-families may have originated from the Liliaceæ along quite independent lines.

SUMMARY

The paper deals with floral organogeny and anatomy, structure and development of the ovules and embryo-sac, development of the anther, meiosis and pollen formation in *Polianthes tuberosa*. In the end the bearing of these observations on the origin of the inferior ovary, genetical constitution of the species, cause of sterility, origin of the linear tetrad and classification of the Liliifloræ is discussed.

It is found that the traces of the various floral parts separate out from the stele of the floral axis below the ovary. In the wall of the ovary the bundles of the outer and inner whorls of perianth leaves, stamens and carpels are quite distinct from one another. This shows that the inferior ovary has originated as the result of adhesion between the various floral whorls.

The ovules are anatropous, bitegmic and are borne along the margins of each carpel. There is a single primary archesporial cell, which cuts off a wall cell. The megaspore-mother cell gives rise to a linear or T-shaped tetrad of megaspores. The development of the embryo-sac is normal. Very rarely antipodals may be 2-nucleate. The endosperm probably develops according to the *Helobiales*-type.

The primary archesporium in each anther-lobe is hypodermal and consists of 2-3 rows of cells. The tapetum takes its origin from the innermost parietal layer. The nucleus of each tapetal cell divides twice to form 4 nuclei. The grand-daughter nuclei fuse in pairs, so that the mature tapetal cells generally show two tetraploid nuclei. The two parietal layers just below the epidermis develop fibrous thickenings. The endothecium is thus mostly 2-layered.

The haploid complement consists of 5 long and 25 small chromosomes. In the diploid set two pairs of chromosomes are associated with the organization of the nucleolus. The vacuolation of the nucleoli, their number and the forces causing their fusion during the prophase of meiosis are discussed. The small bivalents during I and II metaphase show secondary pairing. Groups of 2, 3 or rarely 4 bivalents are observed.

The pollen grains are formed according to the *Successive*-type. The tetrads may be linear, T-shaped or bilateral. The form of the tetrads depends upon the form of the mother cells. The mature pollen grains are 2-nucleate and 1-furrowed.

From the number of the nucleolar chromosomes, number of nucleoli and the secondary pairing of chromosomes, it is inferred that *Polianthes tuberosa* is probably a secondarily balanced allotetraploid.

Cytological observations support Hutchinson's classification of monocotyledons for bringing the Yuccae and Agavoideae under one family Agavaceae. His classification of the old tribe Agavoideae into Agaveae and Poliantheae, however, appears to be unnatural. It would be better to divide Agavoideae into two groups, one including only the Australian genus *Doryanthes* and the other comprising the remaining 6 genera (*Agave*, *Furcraea*, *Beschorneria*, *Polianthes*, *Prochnyanthes* and *Pseudobravia*), which are all American.

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DESMIDS FROM KODAIKANAL, SOUTH INDIA*

BY M. O. P. IYENGAR, M.A., PH.D. (LOND.), F.L.S.

AND

MISS B. VIMALA BAI, B.Sc. (HONS.), M.Sc. (MADRAS)

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THE number of papers dealing with systematic accounts of Desmids from various parts of India is rather few. Wallich's (1860) paper on the Desmidiaceæ from lower Bengal was the first systematic account of Desmids from India. In this paper he described nearly 140 species collected in the neighbourhood of Raneegunge—a place about 120 miles North-West of Calcutta. Hobson in 1863 published an account of two Desmids from Mahabuleswar in the Bombay Presidency. Grunow in 1865 recorded 14 Desmids from the Island of Banka near Singapore. In 1873 Zeller recorded a few Desmids from Burma. Lagerheim (1888) recorded about 52 species from Bengal. Joshua (1885, 1886) recorded about 188 species and varieties from Rangoon. Turner (1893) gave an account of about 540 forms from North India based mainly on Wallich's extensive collections and notes. Borge (1899) recorded several forms from Bengal, Ceylon and Singapore. W. and G. S. West (1897) described a few desmids from Singapore. Schmidle in 1900 gave an account of 26 Desmids collected by Hansgirg in Bombay and the neighbourhood. In 1902, W. and G. S. West recorded about 246 species from Ceylon and Fritsch in 1907 recorded some Desmids from the same country. In 1907 W. and G. S. West gave a further account of 148 species, chiefly from Burma and a few from Bengal. In 1926 Carter recorded about 100 species from North-Western Himalayas, North-Western Frontier and Satpura Hills in the Central Provinces. About 121 species were recorded by P. Brühl and K. Biswas (1926) from the Loktak lake in the Manipur State.

All these records are from North India, Burma or Ceylon. Practically no work appears to have been done so far on the Desmidiaceæ of South India. The Desmids which form the subject of this paper were collected in 1921, 1923, 1933 and 1936 from Kodaikanal, a hill station in South India, with an elevation of about 7-8000 feet above the sea-level. The climate is sub-tropical and nearly temperate. Most of the forms described in this paper are planktonic and collected from the Kodaikanal lake and a few were collected from a swamp in Kodaikanal. The material was preserved in 4% formalin.

* From the University Botany Laboratory, Madras.

On the whole 35 forms are recorded in this paper, representing 13 genera. Of these 7 are new varieties and one a new form. Of the remaining 27 forms, 8 are new to India, Burma and Ceylon.

Systematic

Genus *Gonatozygon* De Bary 1856

1. *Gonatozygon Kinahani* (Arch.) Rabenh.

(Figs. 1, 2a, 2b)

Gonatozygon Kinahani Rabenhorst, *Flor. Europ. Alg.*, III, 1868, p. 156; Cooke, *Brit. Desmids*, 1887, p. 3. Pl. 1, Fig. 3; De Toni, *Syll. Alg.*, I, 1889, p. 802; W. and G. S. West, *Mon. Brit. Desmidiaceae*, I, 1904, p. 35, Pl. 2, Figs. 1-3.

Gonatozygon leioderium Turner, 1893, p. 24, Pl. 20, Fig. 5.

Cells mostly single, sometimes in chains of two or more cells; 10-19 times longer than broad; cylindrical; apices truncate slightly dilated. Cell wall perfectly smooth; pyrenoids 6-10 in each chloroplast.

Dimensions :

Length	124-259.5 μ
Breadth	10.9-14 μ

Hab.—Planktonic in Kodaikanal Lake.

The present form agrees with *Gonatozygon leioderium* Turner (Turner, 1893) in general appearance and dimensions. Turner's Desmid was collected at Nilgiris, another hill station in South India, of the same elevation as Kodaikanal, viz., 7-8,000 ft. above the sea-level. With regard to Turner's *G. leioderium*, W. and G. S. West (1895, p. 65) state that it is "very probably an *Oedogonium*". But Turner's figure shows clearly that it is not an *Oedogonium* as suspected by West and West, since the cells are swollen at both the ends as is characteristic of *Gonatozygon* and not at one end only as in *Oedogonium*. There appears to be therefore no sufficient reason for considering the alga an *Oedogonium* and not a *Gonatozygon*.

Genus *Netrium* Nägeli 1849

2. *Netrium digitus* (Ehrbg.) Itzigs and Rothe.

(Figs. 5, 6)

Penium digitus Ralfs, *Brit. Desm.*, 1848, p. 150, Pl. 25, Fig. 3; Rabenhorst, *Flor. Europ. Alg.*, III, 1868, p. 118; Delponte, *Desm. Subalp.*, 1877, p. 86, Pl. 15, Figs. 50 and 51; West and West, *Freshw. Alg. Ceylon*, 1902, p. 134.

Penium digitus forma *rectum* Turner, *Freshw. Alg. E. India*, 1893, p. 18, Pl. 1, Fig. 27.

Netrium digitus West, W. and G. S., *Mon. Brit. Desm.*, I, 1904, p. 64, Pl. 6, Figs. 14-16; Smith, *Wisconsin phytoplankton*, pt. II, 1924, p. 6, Pl. 52, Fig. 5; Krieger, *Die Desmidiaceen der Deutsch Limn. Sunda Expedition*, 1932, p. 158, Pl. 3, Fig. 4.

Cells single, large, 3-4 times longer than broad, unconstricted, elliptic oblong, gradually attenuated towards the apices. Apex round, cell wall smooth. Chloroplast with eight longitudinal plates deeply notched at the free ends.

Dimensions :

Length	155-203 μ
Breadth at the middle	40-44 μ
Breadth at the apex	18-23.7 μ

Hab.—Kodaikanal lake.

Genus *Closterium* Nitzsch 1817

3. *Closterium libellula* Focke var. *pulneyensis* var. nov.

(Figs. 7, 8a, 8b)

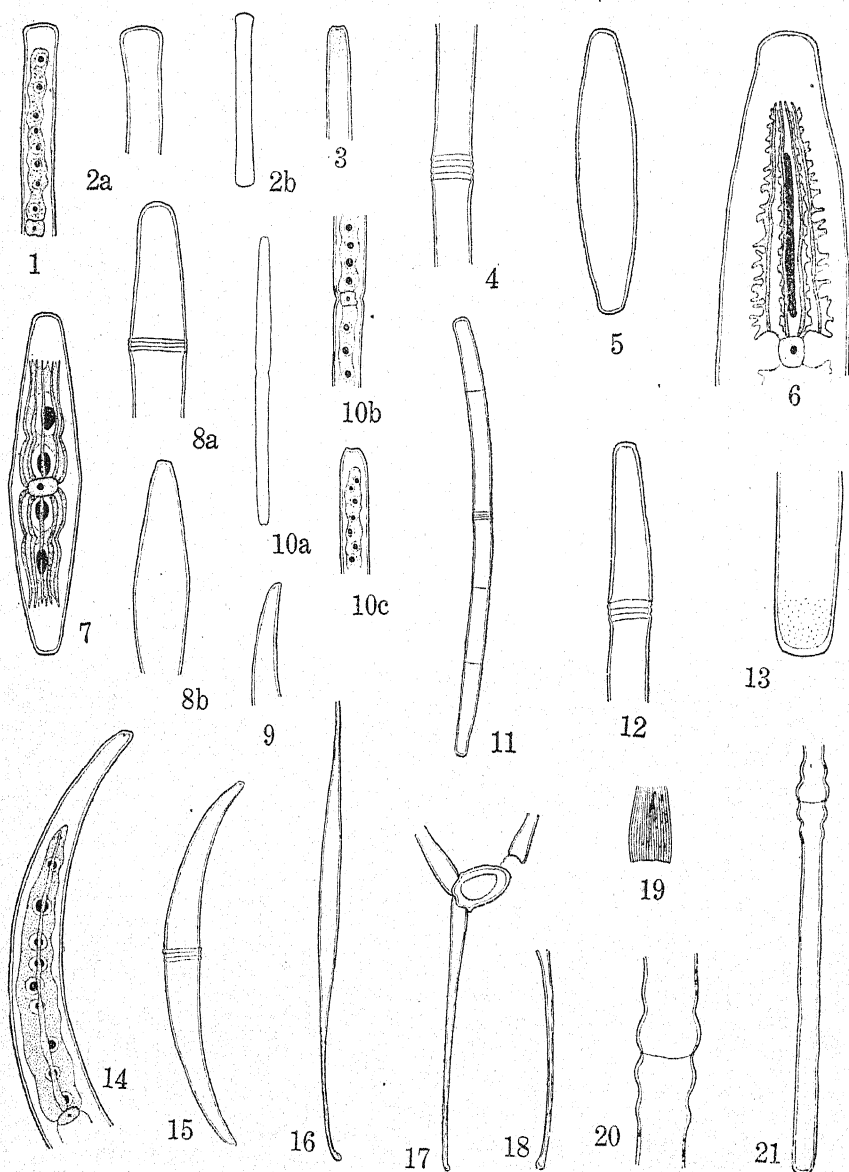
Cells single, size variable, generally large, 4-5 times longer than broad, not constricted, gradually attenuated from the middle towards the apices. Apex broadly rounded. Cell wall smooth, colourless or slightly brownish, with girdles very faintly visible at the isthmus. Each chloroplast slightly notched or constricted and not separated, with about 8 long plates. Pyrenoids two or three in each semicell.

Dimensions :

Length	73-122.6 μ
Breadth at the middle	16.4-27 μ
Breadth at the apex	8.5-13.6 μ

Hab.—Kodaikanal Lake.

This at first sight appears to be a species of *Netrium*. But a treatment with concentrated KOH solution shows that the cell wall is made up of two pieces. A few girdle bands also are seen very faintly after this treatment. So this belongs to the Placodermæ. This form comes very near *Closterium libellula* var. *interruptum* (Krieger, 1935, p. 256, Pl. 12, Fig. 6) in general shape and measurements but the chloroplasts of the latter are completely divided into two in each semicell, while in the present form it is only deeply notched in the middle. This kind of a notched chloroplast is not seen in any of the varieties. The desmid appears therefore to be a new variety of *Cl. libellula*.



Figs. 1-21. Fig. 1. *Gonatozygon Kinahani*, chloroplast and nucleus ($\times 410$). Fig. 2a. *Gonatozygon Kinahani*, end of the cell ($\times 410$). Fig. 2b. *Gonatozygon Kinahani*, single cell ($\times 190$). Fig. 3. *Pleurotaenium minutum* var. *gracile*, part of cell showing punctae ($\times 410$). Fig. 4. *Closterium didymotocum* var. *annulatum* var. nov., girdle bands at the isthmus ($\times 410$). Fig. 5. *Netrium digitus*, single cell ($\times 190$). Fig. 6. *Netrium digitus*, chloroplast and the nucleus ($\times 410$). Fig. 7. *Closterium*

4. *Closterium Kützingii* Breb.

(Figs. 16, 17, 18, 19)

Wolle, *Desm. U.S.*, 1884, p. 47, Pl. 8, Fig. 8; Cooke, *Brit. Desm.*, 1887, p. 134, Pl. 5, Fig. 3; De Toni, *Syll. Alg.*, 1889, p. 850; Turner, *Freshw. Alg. E. India*, 1893, p. 22, Pl. 1, Fig. 12; Nordst., *Index Desm.*, 1896, p. 152; W. and G. S. West, *Mon. Brit. Desm.*, I, 1904, p. 186, Pl. 25, Figs. 6-11; Bernard, *Protococcaeées et Desm.*, 1908, p. 64, Figs. 52-54; Smith, *Wisconsin phytoplankton*, pt. II, 1924, p. 12, Pl. 53, Fig. 6.

Cells single, medium size; median part fusiform; outer and inner margins almost equally convex, attenuated towards each extremity into long processes; apices slightly incurved, round and often slightly swollen; cell wall colorless or straw coloured, striated, about 15 striae visible across the cell. Pyrenoids 5-10 in each semi-cell.

Dimensions:

Length	296-456.7 μ
Breadth at the middle	9-14 μ
Breadth at the apex	2.5-3.6 μ

Hab.—Kodaikanal Lake.

This form is slightly smaller (narrower) especially in breadth than those described by W. and G. S. West and Smith, but agrees with Turner's form in all measurements. This form has 5-10 pyrenoids whereas in the type the number is 4-5 in each chloroplast.

5. *Closterium Dianæ* Ehrenberg

(Figs. 9, 14, 15)

Closterium acuminatum Rabenh., *Flor. Europ. Alg.*, III, 1868, p. 133; Wolle, *Desm. U.S.*, 1884, p. 44; De Toni, *Syll. Alg.*, 1889, p. 840.

Closterium Dianæ Ralfs., *Brit. Desm.*, 1848, p. 168, Pl. 28, Fig. 5; Rabenh., *Flor. Europ. Alg.*, III, 1868, p. 133; Cooke,

libellula var. *pulneyensis* var. nov., single cell with chloroplasts ($\times 410$). Fig. 8a. *Closterium libellula* var. *pulneyensis*, girdle bands seen clearly after treatment with con. KOH ($\times 410$). Fig. 8b. *Closterium libellula* var. *pulneyensis* ($\times 410$). Fig. 9. *Closterium Dianæ*, tip of the cell ($\times 410$). Figs. 10 a-c. *Pleurotanium minutum* var. *gracile*. a, single cell ($\times 410$); b, showing chloroplast and nucleus ($\times 410$); c, showing chloroplast at the end of the cell ($\times 410$). Fig. 11. *Closterium didymotocum* var. *annulatum* var. nov., single cell ($\times 190$). Fig. 12. *Closterium didymotocum* var. *annulatum* var. nov., girdle bands in the middle of a semi-cell ($\times 410$). Fig. 13. *Pleurotanium Trabecula*, tip of the cell with pores ($\times 410$). Fig. 14. *Closterium Dianæ*, showing chloroplast and nucleus ($\times 410$). Fig. 15. *Closterium Dianæ*, single cell with girdle bands ($\times 410$). Fig. 16. *Closterium Kützingii*, single cell ($\times 190$). Fig. 17. *Closterium Kützingii*, zygote with empty semi-cells ($\times 190$). Fig. 18. *Closterium Kützingii*, tip of the cell ($\times 410$). Fig. 19. *Closterium Kützingii*, striations on the cell-wall ($\times 410$). Fig. 20. *Pleurotanium Trabecula*, portion at the isthmus ($\times 410$). Fig. 21. *Pleurotanium Trabecula*, part of the single cell ($\times 190$).

Brit. Desm., 1887, p. 26, Pl. 23, Fig. 3; Nordst., *Index Desm.*, 1896, p. 104; W. and G. S. West, *Freshw. Alg. Ceylon*, 1902, p. 140; W. and G. S. West, *Mon. Brit. Desm.*, I, 1904, p. 130, Pl. 15, Figs. 1-6; Krieger, *Die Desmidiaceen der Deutsch. Limn. Sunda Expedition*, 1932, p. 160, Pl. 4, Fig. 4.

Cells of medium size, 10-12 times longer than their diameter, fairly well curved, outer margin about 110-125° of arc, inner margin scarcely tumid gradually and gracefully attenuated towards the apices, dorsal margin at each apex obliquely truncate and slightly thickened. Cell wall smooth and of a reddish brown color. Chloroplasts obscurely ridged, 7-10 pyrenoids in a single row.

Dimensions :

Length or distance between the

apices 159-246.5 μ

Breadth 16-23.7 μ

Breadth at the apex 5 μ

Hab.—Kodaikanal Lake.

This is slightly smaller than the European form and resembles *Cl. Dianae* var. *arcuatum* in measurements. It differs from the type in having 7-10 pyrenoids while in the type only 5-6 pyrenoids are present. W. and G. S. West, however, have shown 7 pyrenoids in one of their figures (W. and G. S. West, *Mon. Brit. Desm.*, I, 1904, Pl. 15, Fig. 4).

6. *Closterium didymotocum* Corda

var. *annulatum* var. nov.

(Figs. 4, 11, 12)

Cells single, fairly big, 22-24 times longer than their diameter; outer margin about 25-35° of arc; inner margin very slightly concave, gradually and very slightly attenuated towards the apices. Apex broad and with rounded angles, truncate; cell wall smooth and brownish. Chloroplasts ridged, with 7-9 pyrenoids.

Dimensions :

Length 297-333 μ

Breadth 12-14 μ

Breadth at the apex 8 μ

Hab.—Kodaikanal Lake.

In the fully developed cell, the wall is brownish and girdle bands are also seen well. The dimensions of the cell are very much smaller than those of the type (W. and G. S. West, *Mon. Brit. Desm.*, I, 1904, Pl. 12, p. 116, Figs. 1-5) and several annular thickenings are formed. But the apex of the cell is not thickened or dark as in the type and in this respect the form comes near *Cl. didymotocum* var. *asperulum* op. cit., Pl. 12, Figs. 11-13) but the cell wall differs from that of var. *asperulum*, in being smooth and colored. The minute asperulate type of cell wall as seen in the var. *asperulum* is also absent here.

Again this comes near *Cl. silesiacum* Grönblad (*Beitrag zur Kenntnis der Desmidiaceen Schlesiens*, 1926, p. 10, Pl. 1, Figs. 3-5) and agrees in measurements especially in breadth, but his figures do not show the peculiar annular markings on the cells. Hence it is best to keep it as a new variety of *Cl. didymotocum*, which may be named var. *annulatum*.

Genus *Pleurotænium* Nägeli 1849

7. *Pleurotænium Trabecula* (Ehrbg.) Nägeli

(Figs. 13, 20, 21)

Docidium Trabecula Wolle, *Desm. U.S.*, 1884, p. 48, Pl. 9, Figs. 2-4 and Pl. 11, Figs. 1-7; Turner, *Freshw. Alg. E. India*, 1893, p. 38.

Pl. Trabecula West, W. and G. S., *Mon. Brit. Desm.*, I, 1904, p. 209, Pl. 30, Figs. 11-13; Smith, *Wisconsin phytoplankton*, pt. II, 1924.

Cells big, semi-cells with one basal inflation and a slight second undulation above it gradually attenuated towards the apex. Lateral margins almost straight, apices rounded, destitute of tubercles. Cell wall punctate.

Dimensions.

Length	464-551 μ
Breadth at the isthmus	20-25.6 μ
Breadth at the apex	14-20 μ
Isthmus	14-18 μ

Hab.—Planktonic in Kodaikanal Lake.

8a. *Pleurotænium minutum* Delp. var. *gracile* Wille

(Figs. 3, 10a, 10b, 10c)

W. Krieger, *Die Desmidiaceen der Deutsch. Limn. Sunda Expedition*, 1932, p. 167, Pl. 6, Fig. 7.

Pleurotænium (?) *minutum* (Ralfs) Delp. var. *gracile* Wille, De Toni, *Syll. Alg.*, 1889, I, p. 905.

Penium minutum (Ralfs) Cleve var. *gracile* Wille, W. and G. S. West., *Mon. Brit. Desm.*, I, 1904, pp. 103-4, Pl. 10, Fig. 6.

Cells single, elongated with parallel walls and a very slight constriction in the middle, 16-20 times as long as broad; apex flat with a small depression in the middle, breadth uniform throughout except near the tip where it becomes slightly narrower; cell-wall very minutely punctate.

Dimensions :

Length	170-198.5 μ
Breadth	9-14.3 μ
Breadth at the apex	6.8 μ

Hab.—Planktonic in Kodaikanal Lake.

8b. *Pleurotaenium Kayei* Rabenh.

(Figs. 22, 31)

Docidium horridum Borge, *Austral. Susswasser-chlorophyceen*, 1896, p. 28, Pl. 4, Fig. 55.

Pleurotaenium Kayei Rabenh., *Flor. Europ. Alg.*, III, 1868, p. 144; Gutwinski, *De algis a Dre M. Raciborski anno 1899 in insula Java collectis*, 1902, p. 587, Pl. 37, Fig. 25; W. and G. S. West, *Freshw. Alg. Ceylon*, 1902, p. 141, Pl. 18, Figs. 33-34.

Cells big, 4-5 times longer than their breadth (with spines), semi-cells with spinose margins caused by always four rings of prominent double-headed spines, gradually tapering from the base to the apex; apex slightly dilated, truncate furnished with a peripheral ring of about 10 spine-like projections. Cell-wall smooth or sparsely punctate, punctae not quite distinct.

Dimensions :

Length	217-275.5 μ
Breadth at isthmus with spines	54.9-58.5 μ
Breadth at isthmus without spines	40-47.5 μ
Breadth at the apex with spines	43.9-51 μ
Breadth at the apex without spines	29-32.9 μ
Isthmus	21.9-25 μ

Hab.—In a swamp at Kodaikanal.

Gutwinski (1902) has shown 5 and 6 whorls of spines in his figures while Borge (1896) gives only four rings of spines. The number of spines seems to be variable.

9. *Pleurotaenium tessellatum* (Joshua) Lagerh.var. *bulbosum* Krieger

(Figs. 23, 24)

Krieger, *Die Desmidiaceen der Deutsch. Limn. Sunda Expedition*, 1932, p. 168, Pl. 6, Fig. 11.

Cells single, large, well constricted with sides slightly attenuated towards the apices; basal inflation slight; apex truncate with short spine-like projections. Cell-wall with about 7-10 transverse rings of irregular quadrangular projecting areas, areas being small at the base and elongated at the apex.

Dimensions :

Length	217.5-304.5 μ
Breadth at the base	34-44.9 μ
Breadth at the apex	25-31 μ
Isthmus	21-25.6 μ

Hab.—In a swamp at Kodaikanal.

This form shows a close resemblance to *Pleurotaenium trochiscum* var. *tuberculatum* (Smith, *Wisconsin phytoplankton*, pt. II, 1924, p. 17,

Pl. 55, Fig. 33) but as pointed out by Krieger (1932), *Pl. trochiscum* is more cylindrical, whereas the present form is somewhat narrowed towards the apex. The spines at the apex in the present form are again more robust than in *Pl. trochiscum* var. *tuberculatum*. But the difference between these two species appears to be very very small.

Genus *Euastrum* Ehrenbg. 1832

10. *Euastrum sinuosum* Lenorm.

(Fig. 25)

Ralfs, *Brit. Desm.*, 1848, p. 85; Cooke, *Brit. Desm.*, 1887, p. 71, Pl. 34, Fig. 3; Nordst., *Index Desm.*, 1896, p. 235; W. and G. S. West, *Freshw. Alg. Ceylon*, 1902, p. 148; W. and G. S. West, *Mon. Brit. Desm.*, II, 1905, p. 20, Pl. 36, Fig. 1.

Cells deeply constricted, sinus narrowly linear with a dilated extremity; semi-cells three lobed; polar lobe prominent and outstanding; angles rounded; apex with a narrow median incision; lateral lobes bilobulate, lobules separated by a widely open sinus, rounded lobules, upper not projecting so far as the lower, the margin of the lower lobule slightly crenate; semi-cells with three prominent protuberances across the base, and two across the centre. Cell wall punctate. A small but prominent scrobiculation in each of the protuberance.

Dimensions :

Length	73-86 μ
Breadth at the centre	40-47.5 μ
Breadth at the apex	16-23 μ
Isthmus	10.9-12.8 μ

Hab.—In a swamp at Kodaikanal.

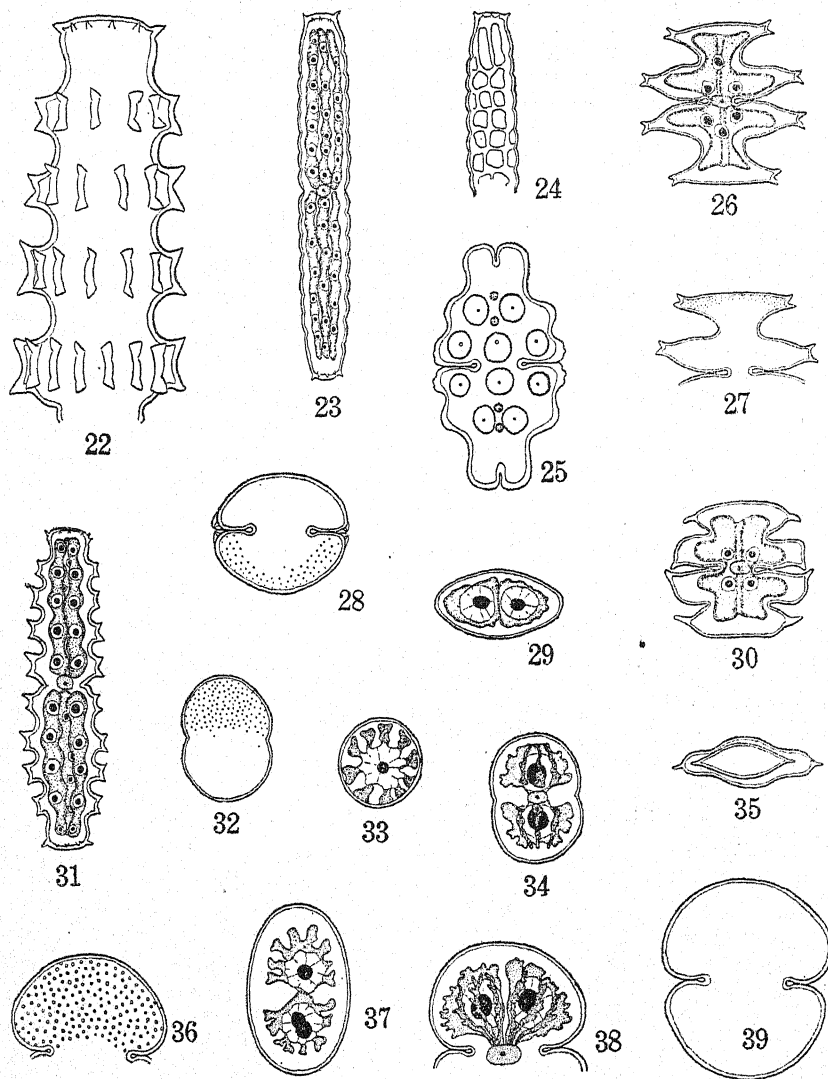
The crenate margin of the lower lobule and the presence of a small prominent scrobiculation in the centre of the protuberances are not seen in the type. In the presence of scrobiculations this resembles *E. sinuosum* var. *ceylanicum* West and West (W. and G. S. West, *Freshw. Alg. Ceylon*, 1902, p. 148, Pl. 19, Fig. 6). But, as only one or two specimens of the present form were found in the material, and since the side views of these individuals could not be obtained, it was decided to leave it for the present under the type itself until more material is available.

Genus *Micrasterias* Agardh 1827

11. *Micrasterias pinnatifida* (Kutz.) Ralfs.

(Figs. 26, 27)

Ralfs, *Brit. Desm.*, 1848, p. 77, Pl. 10, Fig. 3; Wolle, *Desm. U.S.*, 1884, p. 116, Pl. 37, Figs. 7-8; Cooke, *Brit. Desm.*, 1887, p. 54, Pl. 20, Fig. 3; Turner, *Freshw. Alg. E. India*, 1893, p. 88, Pl. 5, Fig. 3; W. and G. S. West, *Mon. Brit. Desm.*, II, 1905,



Figs. 22-39. Fig. 22. *Pleurotænium Kayei*, single semi-cell showing spines ($\times 410$). Fig. 23. *Pleurotænium tessellatum* var. *bulbosum*, single cell with chloroplasts ($\times 190$). Fig. 24. *Pleurotænium tessellatum* var. *bulbosum*, semi-cell with quadrangular areas ($\times 190$). Fig. 25. *Euastrum sinuosum*, single cell ($\times 410$). Fig. 26. *Microsterias pinnatifida*, single cell with chloroplasts ($\times 410$). Fig. 27. *Microsterias pinnatifida*, portion of the cell with pores ($\times 410$). Fig. 28. *Cosmarium obsoletum*, single cell with pores and basal mamillæ with pore canals at the base of semi-cells ($\times 410$). Fig. 29. *Cosmarium obsoletum*, vertical view with chloroplasts ($\times 410$). Fig. 30. *Microsterias incisa* var. *Wallichiana*, single cell with chloroplasts ($\times 410$). Fig. 31. *Pleurotænium Kayei*, single cell with

p. 80, Pl. 41, Figs. 7-11; Smith, *Wisconsin phytoplankton*, pt. II, 1924, p. 41, Pl. 59, Figs. 1-2; Brühl and Biswas, *Alg. of Loktak Lake*, 1926, p. 279, Pl. 6, Fig. 51.

Cells small, slightly broader than long, deeply constricted, sinus open, isthmus very narrow. Semi-cells 3 lobed polar lobe, flattened or slightly convex, widely spreading extremities narrower than those of lateral lobes, ends bifid, lateral lobes horizontally disposed, semifusiform in shape with bifid ends. Incisions between lateral lobes and polar lobe very broad. Cell wall minutely punctate.

Dimensions :

Length	47-51.3 μ
Breadth	49-54.7 μ
Breadth of the polar lobe	38-46 μ
Isthmus	8-10 μ

Hab.—Planktonic in Kodaikanal Lake.

The Kodaikanal form seems to be slightly smaller than the type.

12. *Micrasterias incisa* (Breb.) Ralfs.

var. *Wallichiana* Turner

(Figs. 30, 35)

Turner, *Freshw. Alg. E. India*, 1893, p. 89, Pl. 6, Fig. 52; Brühl and Biswas, *Alg. of the Loktak Lake*, 1926, p. 280, Pl. 6, Figs. 7 and 9.

Cells small, little broader than long, deeply constricted, sinus narrow and opening out; isthmus narrow; semicells three lobed, polar lobe entire, broadly trapezoidal, outer margins converging towards the apex, ending in small spines at the end; lateral lobes horizontal, trapezoidal, ending in short spines towards the sinus; incision between the polar lobe and lateral lobes broad; vertical view oblong elliptic. Cell wall minutely punctate.

Dimensions :

Length	38-41 μ
Breadth	45-51 μ
Isthmus	7-11 μ
Polar lobe	34-40 μ
Thickness	11 μ

Hab.—Planktonic in Kodaikanal Lake.

chloroplasts ($\times 190$). Fig. 32. *Cosmarium globosum*, single cell with pores ($\times 410$). Fig. 33. *Cosmarium globosum*, vertical view with chloroplasts ($\times 410$). Fig. 34. *Cosmarium globosum*, single cell with chloroplasts ($\times 410$). Fig. 35. *Micrasterias incisa* var. *Wallichiana*, vertical view ($\times 410$). Fig. 36. *Cosmarium pachydermum* var. *indicum* var. nov. semi-cell with big pores ($\times 410$). Fig. 37. *Cosmarium pachydermum* var. *indicum* var. nov., vertical view with chloroplasts ($\times 410$). Fig. 38. *Cosmarium pachydermum* var. *indicum* var. nov., semi-cell showing chloroplasts ($\times 410$). Fig. 39. *Cosmarium pachydermum* var. *indicum* var. nov., single cell ($\times 410$).

The measurements of the desmid agree with those given by Turner except that it is slightly shorter in length. The length given by Turner is 48–53 μ whereas here it is only 38–41 μ .

Genus *Cosmarium* Corda 1834

13. *Cosmarium moniliforme* (Turp.) Ralfs.

forma *punctata* Lagerh.

(Figs. 40, 41, 42, 43)

W. and G. S. West, *Mon. Brit. Desm.*, III, 1908, p. 22, Pl. 87, Fig. 4.

Cells single, deeply constricted, sinus widely open, usually acute; semi-cells circular or subcircular; side views of semi-cells almost circular, verticle view circular. Cell wall punctate. One axile chloroplast in each semi-cell with a central pyrenoid and six radiating plates.

Dimensions :

Length	40–47 μ
Breadth	21–27 μ
Isthmus	5–7.6 μ

Hab.—Planktonic in Kodaikanal Lake.

14. *Cosmarium moniliforme* (Turp.) Ralfs

forma *panduriformis* Heimerl

(Figs. 46, 47, 48)

Dysphinctum inferum Turner, *Freshw. Alg. E. India*, 1893, p. 40, pl. 1, Fig. 21.

C. moniliforme forma *panduriformis* W. and G. S. West, *Mon. Brit. Desm.*, III, 1908, p. 22, Pl. 67, Figs. 8–9.

Cells single, very small, slightly constricted; isthmus broad with an obtusely rounded sinus; semi-cells subcircular, vertical view circular, cell wall smooth. Chloroplasts axile, one in each semi-cell with a central pyrenoid and about 6 to 7 radiating vertical plates.

Dimensions :

Length	17–19.6 μ
Breadth	11–12.8 μ
Isthmus	8–10 μ

Hab.—Planktonic in Kodaikanal Lake.

This comes near *C. pseudarctum* (W. and G. S. West, *Mon. Brit. Desm.*, III, 1908, p. 32, Pl. 68, Figs. 12–14) in general shape and size but the four radiating cruciately disposed lobes of the chloroplast are not seen in the present form. This also shows some resemblance to *C. connatum* (W. and G. S. West, *Mon. Brit. Desm.*, III, 1908,

p. 25, Pl. 76, Figs. 15-17) in shape but the present form is much smaller and has a smooth wall.

15. *Cosmarium obsoletum* (Hantzsch) Reinsch

(Figs. 28, 29)

Cosmarium palustre Turner, *Freshw. Alg. E. India*, 1893, p. 60, Pl. 8, Figs. 65 and 64; Pl. 9, Fig. 29.

C. obsoletum subsp. *palustre*. Brühl and Biswas, *Alg. of the Loktak Lake*, 1926, p. 285, Pl. 9, Figs. 91-92.

C. obsoletum Nordst., *Index Desm.*, 1896, p. 186, W. and G. S. West, *Freshw. Alg. Ceylon*, 1933, p. 164; W. and G. S. West, *Mon. Brit. Desm.*, II, 1905, p. 133, Pl. 56, Figs. 1-3.

Cells single, medium size, a little broader than long, deeply constricted, sinus narrowly linear with a dilated apex; semi-cells semielliptic; basal angles submamillate with a small pore or canal; apex convex or slightly flat. Vertical view elliptic slightly attenuated towards poles. Cell wall punctate. Chloroplasts axile, each with two pyrenoids.

Dimensions :

Length	36-40 μ
Breadth	42-45.7 μ
Isthmus	18-25 μ
Thickness	23.9 μ

Hab.—Planktonic in Kodaikanal Lake.

This agrees in dimensions with the smaller European form but shows a mamillate thickening through which the canal referred to in the large tropical forms is also seen (W. and G. S. West, *Mon. Br. Desm.*, 1905, II, p. 134, Pl. 56, Fig. 4). But the place where this form occurs is not typically tropical but subtropical and nearly temperate.

16. *Cosmarium globosum* Bulnh.

(Figs. 32, 33, 34)

Rabenh. *Flor. Europ. Alg.*, III, 1868, p. 178; Cooke, *Brit. Desm.*, 1887, p. 121, Pl. 43, Fig. 6; Nordst., *Index Desm.*, 1896, p. 130; W. and G. S. West, *Mon. Brit. Desm.*, III, 1908, p. 29, Pl. 68, Figs. 1-2.

Cells small, slightly constricted, sinus rapidly widening from an acute apex; semi-cells subcircular; vertical view circular. Chloroplasts axile, with a central pyrenoid and a number (6-8) of vertically disposed lobes.

Dimensions :

Length	27-31 μ
Breadth	20-22 μ
Isthmus	18-20 μ

Hab.—Planktonic in Kodaikanal Lake.

17. *Cosmarium pachydermum* Lund.var. *indicum* var. nov.

(Figs. 36, 37, 38, 39)

Cells single, large, about $1\frac{1}{2}$ times longer than broad, rather deeply constricted, sinus narrowly linear with a dilated apex; semi-cells widely semielliptic, apices broad, sometimes truncate; side view of the semi-cells subcircular, vertical view elliptic; cell wall punctate with big punctæ. Chloroplasts ridged with two pyrenoids in each semi-cell. In vertical view they are stellate with several vertical ridges.

Dimensions :

Length	62-69.5 μ
Breadth	49-58.5 μ
Isthmus	25-29 μ
Thickness	34-37 μ

Hab.—Planktonic in Kodaikanal Lake.

This form closely resembles *Cosmarium pachydermum* (W. and G. S. West, *Mon. Br. Desm.*, II, 1905, p. 139, Pl. 57, Fig. 7) in general shape, but is a much smaller form with thinner walls. It comes very near *C. pachydermum* var. *æthiopicum* but differs in size, in the shape of semi-cells and also in the absence of minor punctæ between scrobiculations.

Genus *Xanthidium* Ehrenberg 183718. *Xanthidium sexmamillatum* W. and G. S. Westvar. *pulneyensis* var. nov.

(Figs. 53, 57)

Cells fairly big, little longer than broad, without spines, deeply constricted, sinus broadly open; semi-cells transversely elliptic; lateral margin with 3 mamillæ on each side, one apical, one sub-apical, and one median. Six strong spines on the apices of these six mamillæ on each semi-cell. Apical and sub-apical spines curved upwards, the median being almost horizontal. A fourth distinctly marked mamillæ with a short spine on both the lower sides of the semi-cell. Apex mostly straight. Vertical view nearly rhomboidal, sides thickened slightly more yellowish, three asymmetrically disposed spines at each pole. Cell wall punctate; pyrenoids 2 in each semi-cell.

Dimensions :

Length without spines	49-52 μ
Length with spines	75-78 μ
Breadth without spines	40-45 μ
Breadth with spines	68-84 μ
Isthmus	10-12 μ
Thickness	30 μ

Hab.—In a swamp at Kodaikanal.

This form comes very near *X. sexmamillatum* W. and G. S. West (*Freshw. Alg. from Burma*, 1907, p. 211, Pl. 15 Figs. 11, 12) especially in the six spines situated on the apices of six mamillate projections, but differs from it in the presence of two more small, well marked, mamillæ on both the lower sides of the semi-cell, with the beginnings of a very short spine on each. The vertical view of the present form differs from that of *X. sexmamillatum* in being more rhomboidal and in the spines being disposed asymmetrically. In this respect (vertical view) it resembles *X. pseudobengalicum* Grönblad (New Desmids from Finland and North Russia, 1921, p. 50, Pl. 4, Figs. 32, 33) but there are no mamillate projections in *X. pseudobengalicum*.

Genus *Arthrodesmus* Ehrenberg 1838

19. *Arthrodesmus subulatus* Kutz.

(Figs. 44, 49, 50)

A. convergens var. *subulatus* (Kutz.) Rabenh. *Flor. Europ. Alg.*, III, 1868, p. 227.

A. subulatus Kutz., *Species. Alg.*, 1849, p. 176; Wolle, *Desm. U.S.*, 1884, p. 96, Pl. 24, Figs. 11, 12; De Toni, *Syll. Alg.*, 1889, p. 1059; Turner, *Freshw. Alg. E. India*, 1893, p. 133; W. and G. S. West, *Mon. Brit. Desm.*, IV, 1912, p. 109, Pl. 116, Fig. 14; Smith, *Wisconsin phytoplankton*, pt. II, 1924, p. 127, Pl. 85, Figs. 1-3.

Cells single, a little longer than broad (without spines), deeply constricted, sinus widely open, angles of the semi-cells, each furnished with a long stout straight spine. Vertical view elliptic with a long spine at each pole. Cell wall finely punctate. Chloroplasts axile, one in each semi-cell with a central pyrenoid and two deeply forked processes radiating one towards each pole.

Dimensions :

Length	23.5-26.8 μ
Breadth without spines	17-23 μ
Breadth with spines	45-55 μ
Isthmus	3.6-5.4 μ
Thickness	12-14 μ

Hab.—Planktonic in Kodaikanal Lake.

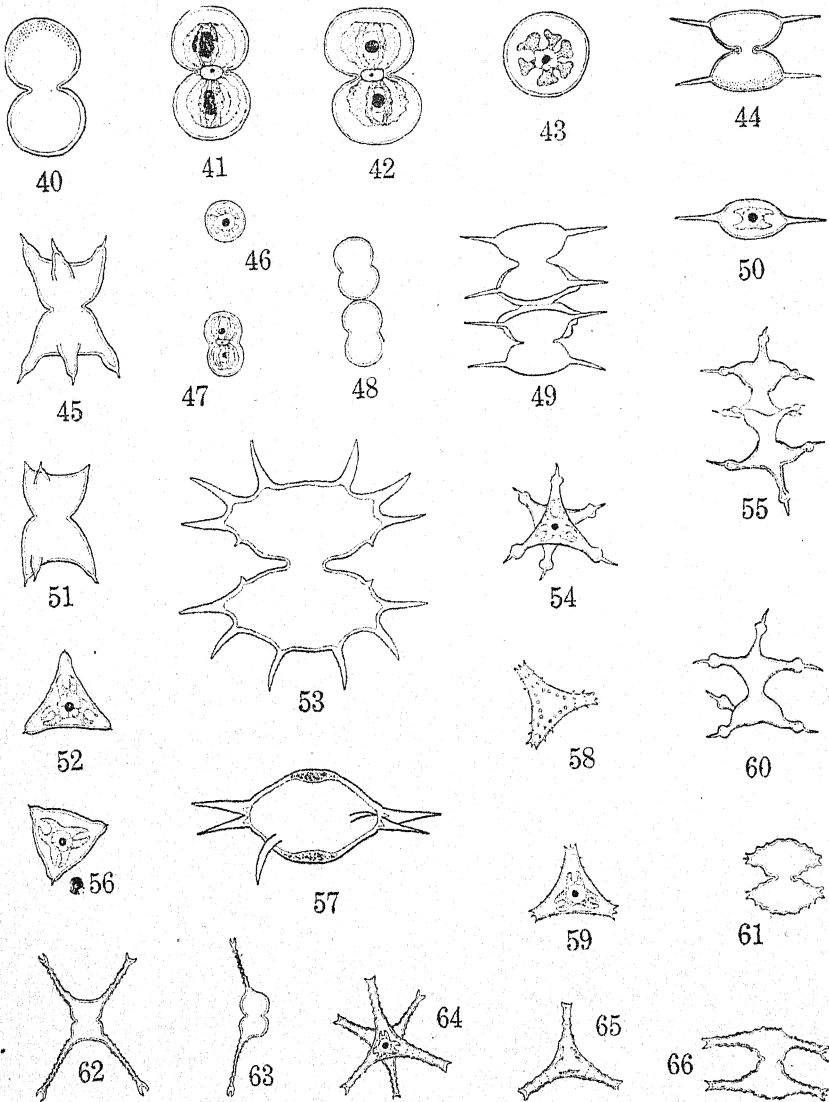
Genus *Staurastrum* Meyen 1829

20. *Staurastrum corniculatum* Lund.

. var. *spinigerum* West

(Figs. 45, 51, 52, 56)

W. and G. S. West, *Mon. Brit. Desm.*, IV, 1912, p. 164, pl. 125, Figs. 19-22.



Figs. 40-66. Fig. 40. *Cosmarium moniliforme* forma *punctata*, single cell with pores ($\times 410$). Fig. 41. *Cosmarium moniliforme* forma *punctata*, semi-cells circular and chloroplasts ($\times 410$). Fig. 42. *Cosmarium moniliforme* forma *punctata*, semi-cells with flattened apices and chloroplasts. ($\times 410$). Fig. 43. *Cosmarium moniliforme* forma *punctata*, vertical view with chloroplasts ($\times 410$). Fig. 44. *Arthrodesmus subulatus*, single cell with pores ($\times 410$). Fig. 45. *Staurostrum corniculatum* var. *spinigerum* West, single cell ($\times 410$). Fig. 46. *Cosmarium moniliforme* forma *panduriformis*, vertical view with chloroplasts ($\times 410$). Fig. 47. *Cosmarium moniliforme* forma *panduriformis*, single cell with chloroplasts ($\times 410$). Fig. 48. *Cosmarium moniliforme* forma *panduriformis*, two daughter cells

Cells small, longer than broad, slightly constricted; semi-cells subcuneate, gradually widened from a broad base; sides very slightly convex, apex straight, angles of semi-cells produced and each furnished with a minute spine. Vertical view triangular with straight sides and 3 small spines at the 3 angles. Chloroplasts two, one in each semi-cell, axile, with a central big pyrenoid and 3 deeply forked processes radiating one into each angle.

Dimensions:

Length	25-29 μ
Breadth	17-21.9 μ
Isthmus	10 μ
Spine	1.7-2 μ

Hab.—Planktonic in Kodaikanal Lake.

This form exhibits variation in having the processes at the angles sometimes tumid (Fig. 45) and sometimes straight (Fig. 51).

21. *Staurastrum Tohopekaligense* Wolle.

(Figs. 79, 86)

St. nonanum Turner, *Freshw. Alg. E. India*, 1893, p. 119, Pl. 15, Fig. 15.

St. Tohopekaligense De Toni, *Syll. Alg.*, 1889, p. 1162; W. and G. S. West, *Freshw. Alg. Ceylon*, 1902, p. 180; W. and G. S. West and N. Carter, *Mon. Brit. Desm.*, V, 1923, p. 178, Pl. 155, Figs. 12-14; Smith, *Wisconsin phytoplankton*, pt. II, 1924, p. 121, Pl. 82, Figs. 8-11.

Cells of medium size, deeply constricted, sinus narrow at first, later widely opening; semi-cells broadly oval; lateral angles produced to form long slender processes; upper part of semi-cells with

after division ($\times 410$). Fig. 49. *Arthrodesmus subulatus*, daughter cells after division showing the outer piece ($\times 410$). Fig. 50. *Arthrodesmus, subulatus*, vertical view showing chloroplasts ($\times 410$). Fig. 51. *Staurastrum corniculatum* var. *spinigerum*, single cell ($\times 410$). Fig. 52. *Staurastrum corniculatum* var. *spinigerum*, vertical view with chloroplasts ($\times 410$). Fig. 53. *Xanthidium sexmamillatum* var. *pulneyensis* var. nov., single cell ($\times 410$). Fig. 54. *Staurastrum unicolorne* var. *gracile* var. nov., vertical view showing chloroplasts ($\times 410$). Fig. 55. *Staurastrum unicolorne* var. *gracile* var. nov., cells in division ($\times 410$). Fig. 56. *Staurastrum corniculatum* var. *spinigerum*, vertical view with chloroplasts ($\times 410$). Fig. 57. *Xanthidium sexmamillatum* var. *pulneyensis* var. nov., vertical view ($\times 410$). Fig. 58. *Staurastrum hexacerum*, vertical view showing spines ($\times 410$). Fig. 59. *Staurastrum hexacerum* vertical view showing chloroplasts ($\times 410$). Fig. 60. *Staurastrum unicolorne* var. *gracile* var. nov., single cell ($\times 410$). Fig. 61. *Staurastrum hexacerum*, single cell ($\times 410$). Fig. 62. *Staurastrum columbetoides*, single cell ($\times 410$). Fig. 63. *Staurastrum columbetoides*, side view of the cell ($\times 410$). Fig. 64. *Staurastrum gracile*, vertical view with chloroplasts ($\times 410$). Fig. 65. *Staurastrum gracile*, vertical view with ridges at the top ($\times 410$). Fig. 66. *Staurastrum gracile*, single cell ($\times 410$).

six sub-apical processes, altogether about 9 in each semi-cell. All processes hollow, ending in two long divergent spines. Vertical view triangular with sides straight, bending only at the angles, produced into long processes, with another pair at each lateral side. Cell wall punctate. Chloroplast axile, with a big central pyrenoid and 6-9 radiating processes, some extending into the processes also.

Dimensions :

Length without spines	32-44.4 μ
Length with spines	72-80 μ
Breadth without processes	27-32.2 μ
Breadth with processes	58-65 μ
Isthmus	10-14 μ

Hab.—Planktonic in Kodaikanal Lake.

The type species has two or three spines at the end of each process. But here the number of spines is always two. This form appears to be same as *St. nonanum* Turner.

22. *Staurastrum unicorn* Turner

var. *gracile* var. nov.

(Figs. 54, 55, 60)

Cells single, small, deeply constricted; semi-cells cuneate or triangular the sides being more convex; angles of the semi-cells produced into smooth processes with capitate ends and short fine spines; the processes before the capitate ends narrow and elongated. Vertical view always trigonal, the lateral sides being mostly flat or occasionally concave. Chloroplasts two, axile, one in each semi-cell with a central axis enclosing a big pyrenoid and three massive deeply forked processes radiating one into each angle.

Dimensions :

Length	21-25.5 μ
Breadth with processes	38-43.9 μ
Isthmus	5-7 μ

Hab.—Planktonic in Kodaikanal Lake.

This Desmid resembles *Staurastrum scolopacinum* (Turner, *Freshw. Alg. E. India*, 1893, p. 107, Pl. 17, Fig. 10) in general appearance, but is more constricted below the swellings. In this latter point it comes near *Staurastrum unicorn* (Turner, *Freshw. Alg. East India*, 1893, p. 107, Pl. 15, Fig. 16). But the dorsal surface of the present form is more flat and the arms are much more elongated and narrowed than in *St. unicorn*. It differs from it again in having the sides either flat or concave in vertical view and also in having the tips much more tumid.

23. *Staurostrum contectum* Turner
var. *inevolutum* Turner.

(Figs. 67, 71)

W. and G. S. West, *Some North American Desmidiæ*.
Trans. Linn. Soc., Series II, B. 1896, p. 257, Pl. 16, Fig. 18.

Cells small, single, broader than long, deeply constricted, sinus acute; semi-cells trapezoidal; bifid and curved spine on each side near the apex, apex broad and flat with two spines at each angle. Vertical view triangular, sides slightly concave. Cell wall minutely punctate; chloroplasts two, one in each semi-cell, axile with a central axis enclosing a single big pyrenoid, three deeply forked processes radiating one into each arm.

Dimensions :

Length	21-29 μ
Breadth with spines	32-40 μ
Isthmus	5-7 μ

Hab.—Planktonic in Kodaikanal Lake.

24. *Staurostrum longibrachiatum* (Borge) Gutwin.
var. *intermedium* var. nov.

(Figs. 77, 80, 81)

Cells single, medium size, fairly constricted, sinus widely open, semi-cells truncate gradually attenuated towards the apex with four big verrucæ at the apex, two big in the centre and two small on either side. Angles of the semi-cells produced into long hollow slender processes with sharply dentate upper and lower margins, ends of the processes bifurcated, a short verruca at the base of the process on each side of the semi-cells. Vertical view elliptic, poles continued into long processes with slightly undulate margins, and about four verrucæ in the top view. Cell wall punctate. Chloroplasts axile with a central big pyrenoid, and four radiating forked processes.

Dimensions :

Length	31-36.6 μ
Breadth with processes	65-95 μ
Breadth without processes	12-16 μ
Isthmus	4-7 μ
Thickness	12-14 μ

Hab.—Planktonic in Kodaikanal Lake.

This form resembles *St. longibrachiatum* (Borge) Gutwin. (Borge, *Austral. Susswass. Chl.*, 1896, p. 15, Pl. 2, Fig. 22; Gutwinski, *De Algis a Dre M. Raciborski anno 1899 in Insula Java coll.*, 1902, p. 605, Pl. 40, Fig. 62) in general appearance and in the number of verrucæ at the apex. Borge has shown only four at the apex. But the sides of the semi-cells have more verrucæ in his as well

as in Gutwinski's figures. In possessing only one at the side of the semi-cell this comes near to *Staurostrum longibrachiatum* var. *pseudanchora* (Krieger, *Die Desmidiaceen der Deutsch. Limn. Sunda Expedition*, 1932, p. 202, Pl. 16, Fig. 3) but Krieger's figure shows more verrucæ at the apex. Hence the present form is best kept as a new variety.

25. *Staurostrum retusum* Turner
var. *punctulatum* Eichl. and Gutwin.

(Figs. 82, 84)

W. and G. S. West, *Freshw. Alg. Ceylon*, 1902, p. 178;
Freshw. Alg. of Burma, 1907, p. 216, Pl. 15, Figs. 30-32.

Cells single, small, deeply constricted, semi-cells pyramidate or trapeziform, angles slightly rounded, lateral margins convex, vertical view triangular, rounded angles and concave sides. Cell wall punctate. Chloroplast axile with a central big pyrenoid and three deeply forked processes radiating one into each arm.

Dimensions :

Length	21-23.7 μ
Breadth	21-23.7 μ
Isthmus	5-6.8 μ

Hab.—Planktonic in Kodaikanal Lake.

26. *Staurostrum gladiusum* Turner

(Figs. 68, 69, 72, 73)

De Toni, *Syll. Alg.*, 1889, p. 1172; Turner, *Freshw. Alg. E. India*, 1893, p. 112, Pl. 17, Fig. 2; W. and G. S. West and N. Carter, *Mon. Brit. Desm.*, V, 1923, p. 57, Pl. 137, Figs. 1-2; Krieger, *Die Desmidiaceen der Deutsch. Limn. Sunda Expedition*, 1932, p. 199, Pl. 15, Fig. 14.

Cells of medium size, about as long as broad or slightly longer than broad, sinus acute and not very widely open; semi-cells elliptic reniform; cell wall uniformly covered with stout spines, more or less arranged in circles and scattered further away. Vertical view triangular, sides slightly concave, angles broadly rounded, about 6-8 spines on each side. Chloroplast axile, one in each semi-cell, with a central big pyrenoid and three deeply forked lobes radiating one into each angle.

Dimensions :

Length with spines	31-34.7 μ
Length without spines	25-31 μ
Breadth with spines	27-32 μ
Breadth without spines	23.7-25.6 μ
Isthmus	5-7 μ

Hab.—Planktonic in Kodaikanal Lake.

This form seems to be slightly smaller than the type.

27. *Staurastrum furcatum* (Ehr.) Breb.

(Figs. 75, 76)

Xanthidium furcatum Ralfs, *Brit. Desm.*, 1848, p. 213.*Staurastrum spinosum* Ralfs, *Brit. Desm.*, 1848, p. 143, Pl. 22, Fig. 28; De Bary, *Conjugaten*, 1858, p. 44.*Staurastrum furcatum* Rabenh., *Flor. Europ. Alg.*, III, 1868, p. 218; Nordst., *Norges Desm.*, 1873, p. 33; Wolle, *Desm. U.S.*, 1884, p. 150, Pl. 40, Figs. 40-41; Cooke, *Brit. Desm.*, 1887, p. 146; De Toni, *Syll. Alg.*, 1889, p. 1153; W. and G. S. West and N. Carter, *Mon. Brit. Desm.*, V, 1923, p. 173, Pl. 155, Figs. 1-4; Smith, *Wiscon. phytopl.*, pt. II, 1924, p. 118, Pl. 83, Figs. 1-3; Krieger, *Die Desmidiaceen der Deutsch. Limn. Sunda Expedition*, 1932, p. 199, Pl. 17, Fig. 11.

Cells small, slightly longer than broad, deeply constricted, sinus acute, each semi-cell with 9 bifid processes, cell wall smooth. Vertical view triangular, angles continued into short processes ending in a spine, sides with a pair of bifid processes on each lateral margin. Chloroplast axile, one in each semi-cell, with a central pyrenoid and 3 forked processes radiating one into each angle.

Dimensions :

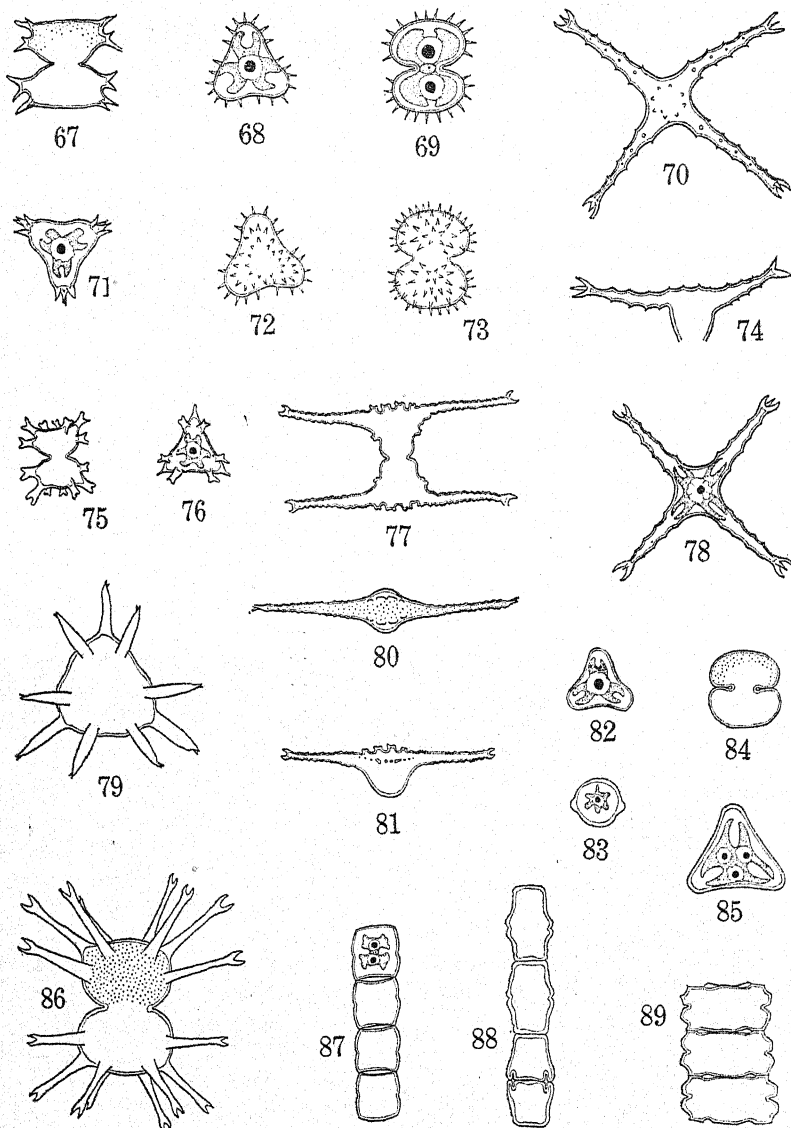
Length with spines	18-29 μ
Length without spines	15-19.5 μ
Breadth with spines	17-25.6 μ
Breadth without spines	11.9-17 μ
Isthmus	5-6.8 μ

Hab.—Planktonic in Kodaikanal Lake.28. *Staurastrum hexacerum* (Ehr.) Wittr.

(Figs. 58, 59, 61)

Staurastrum tricornis Ralfs, *Brit. Desm.*, 1848, p. 134, Pl. 22, Fig. 11, and Pl. 34, Fig. 8a; Delponte, *Desm. subalp.*, 1877, p. 145, Pl. 11, Figs. 48-50; Wolle, *Desm. U.S.*, 1884, p. 126, Pl. 41, Figs. 36-38; Cooke, *Brit. Desm.*, 1887, p. 167, Pl. 53, Fig. 2.*Staurastrum hexacerum* Turner, *Freshw. Alg. E. India*, 1893, p. 125; W. and G. S. West, *Freshw. Alg. Burma*, 1907, p. 218; W. and G. S. West and N. Carter, *Mon. Brit. Desm.*, 1923, V, p. 138, Pl. 142, Figs. 11-14.

Cells small, slightly broader than long, deeply constricted, sinus open; semi-cells sub-triangular, both margins being convex and tapering towards the angles, forming very short processes ending in about 3-4 spines. Cell wall rough with tiny granules arranged in concentric series. Vertical view triangular; lateral margins concave; chloroplast axile with a big central pyrenoid and three deeply forked processes radiating one into each angle.



Figs. 67-89. Fig. 67. *Staurastrum contectum* var. *inevolutum*, single cell showing punctæ ($\times 410$). Fig. 68. *Staurastrum gladiusum*, vertical view with chloroplasts ($\times 410$). Fig. 69. *Staurastrum gladiusum*, single cell with chloroplasts ($\times 410$). Fig. 70. *Staurastrum arachne* var. *pulneyensis* var. nov., vertical view showing spines ($\times 410$). Fig. 71. *Staurastrum contectum* var. *inevolutum*, vertical view showing chloroplasts ($\times 410$). Fig. 72. *Staurastrum gladiusum*, vertical view showing spines

Dimensions :

Length	21.9-23.7 μ
Breadth with processes	21.9-26.4 μ
Breadth without processes	14.6-18 μ
Isthmus	3.6-5.4 μ

Hab.—Kodaikanal Lake.

This form exhibits a certain amount of variation in shape and in the granules. Sometimes the granules appear as very short spinous projections and sometimes they appear only as marginal denticulations.

29. *Staurastrum columbetoides* West and West

(Figs. 62, 63)

W. and G. S. West, *Freshw. Alg. Ceylon*, 1902, p. 186, Pl. 22, Figs. 8-9.

Cells single, small, about $1\frac{1}{4}$ times longer than broad without processes, deeply constricted, sinus narrow and linear; semi-cells truncate pyramidal, sides slightly convex, angles produced into thin delicate, long processes with denticulated margins and bifurcate ends.

Dimensions :

Length with processes	38-47.5 μ
Length without processes	12.8-16.4 μ
Breadth with processes	27-38 μ
Breadth without processes	10-14.6 μ
Isthmus	7-5 μ

Hab.—Planktonic in Kodaikanal Lake.

($\times 410$). Fig. 73. *Staurastrum gladiusum*, single cell with spines ($\times 410$). Fig. 74. *Staurastrum arachne* var. *pulneyensis* var. nov., semi-cell ($\times 410$). Fig. 75. *Staurastrum furcatum*, single cell ($\times 410$). Fig. 76. *Staurastrum furcatum*, vertical view with chloroplasts ($\times 410$). Fig. 77. *Staurastrum longibrachiatum* var. *intermedium* var. nov., single cell ($\times 410$). Fig. 78. *Staurastrum arachne* var. *pulneyensis* var. nov., vertical view showing chloroplasts ($\times 410$). Fig. 79. *Staurastrum Tohopekaligense*, vertical view ($\times 410$). Fig. 80. *Staurastrum longibrachiatum* var. *intermedium* var. nov., vertical view ($\times 410$). Fig. 81. *Staurastrum longibrachiatum* var. *intermedium* var. nov., semi-cell showing the verrucae ($\times 410$). Fig. 82. *Staurastrum retusum* var. *punctulatum*, vertical view with chloroplasts ($\times 410$). Fig. 83. *Gymnozyga moniliformis*, vertical view with chloroplasts ($\times 410$). Fig. 84. *Staurastrum retusum* var. *punctulatum*, single cell with pores ($\times 410$). Fig. 85. *Desmidium Swartzii*, vertical view with chloroplasts ($\times 410$). Fig. 86. *Staurastrum Tohopekaligense*, single cell showing pores ($\times 410$). Fig. 87. *Hyalotheca dissiliens* ($\times 410$). Fig. 88. *Gymnozyga moniliformis*, filament with one cell in division showing the replicate folds ($\times 410$). Fig. 89. *Desmidium Swartzii* ($\times 410$).

30. *Staurostrum arachne* Ralfsvar. *pulneyensis* var. nov.

(Figs. 70, 74, 78)

Cells single, small, fairly constricted; semi-cells cup-shaped with the apices bearing small spines; angles produced into long processes, each process being tipped with about 3 spines and rough with about 5-6 concentric series of denticulations. Vertical view four-sided with concave sides and a circle of eight granules in the centre. Chloroplast axile with four lobes, deeply forked, radiating one into each process.

Dimensions:

Length	25.6-27 μ
Breadth with processes	68-70 μ
Breadth without processes	17-18.7 μ
Isthmus	8.5-9.4 μ

Hab.—Kodaikanal Lake.

The circle of 8 spines seen in the vertical view appears as spines at the apex in side view. In possessing this ring of spines, the Desmid comes very near *St. arachne* var. *arachnoides* (W. and G. S. West and N. Carter, *Mon. Brit. Desm.*, V, 1923, p. 132, Pl. 150, Fig. 3). But it differs from var. *arachnoides* in possessing only 8 spines and being only 4 radiate while var. *arachnoides* has 9-10 verrucæ and is 4-5 radiate. Again 5-6 concentric rows of denticulations in the processes makes the present form quite different from var. *arachnoides* which has the rows much closer and also a large number of rows, about 9-11 or more.

31. *Staurostrum gracile* Ralfs

forma (Figs. 64, 65, 66)

Cells variable, usually small about $1\frac{1}{2}$ times broader than long including the processes, constriction slight as a notch; semi-cells cup-shaped broadening slightly towards the apex which is very slightly convex. Angles produced into long processes tipped with 3 or 4 minute spines and provided with several concentric series of denticulations; processes horizontal or slightly curved. Vertical view always triangular, with the sides straight or slightly concave and the angles produced to form long processes, elongated ridges seen inside the lateral sides running somewhat parallel to it; each ridge appearing slightly constricted in the middle. Chloroplast axile with a central big pyrenoid and three deeply forked processes radiating one into each arm.

Dimensions:

Length	21-24.6 μ
Breadth with processes	29-39.6 μ
Breadth without processes	10-13.6 μ
Isthmus	5-5.9 μ

Hab.—Kodaikanal Lake.

This comes very near *St. gracile* var. *ornatum* (Krieger, *Die Desmidiaceen der Deutsch. Limn. Sunda Expedition*, 1932, p. 200, Pl. 18, Fig. 13) in general outline and in the possession of the ridges in the vertical view. But in var. *ornatum* the ridges are well marked into 6 double-headed distinct verrucae whereas the present form has only long ridges. In addition var. *ornatum* has a small granule at the isthmus on each side of the semi-cell, which is absent here. *St. gracile* forma (Krieger, *op. cit.*, p. 200, Pl. 18, Fig. 12) also resembles this form but the *forma* has more arched arms in the side view and in the vertical view no ridges are present. So the present form appears to be different from the other varieties in possessing the elongated ridges which are double and very slightly divided in the middle. *St. gracile* var. *coronulatum* (W. and G. S. West and N. Carter, *Mon. Brit. Desm.*, V, 1923, p. 100, Pl. 144, Fig. 10) also resembles this but this has not got the ridges but only distinct emarginate processes.

Genus *Hyalotheca* Ehrenberg 1840

32. *Hyalotheca dissiliens* (Sm.) De Breb.

(Fig. 87)

Ralfs, *Brit. Desm.*, 1848, p. 51, Pl. 1, Fig. 1; Delponte, *Desm. subalp.*, 1877, p. 47; Turner, *Freshw. Alg. E. India*, 1893, p. 151; W. and G. S. West, *Freshw. Alg. of Ceylon*, 1902, p. 195; W. and G. S. West and N. Carter, *Mon. Brit. Desm.*, Vol. V, 1923, p. 230, Pl. 161, Figs. 16-27; Krieger, *Die Desmidiaceen der Deutsch. Limn. Sunda Expedition*, 1932, p. 221, Pl. 26, Fig. 11.

Filamentous, cells small, faintly constricted, constriction being a slight concavity in the middle of the lateral margins. Chloroplast axile, with a central pyrenoid and a number of radiating ridges.

Dimensions :

Length	13-17 μ
Breadth	16-18 μ

Hab.—Kodaikanal Lake.

Genus *Desmidium* Agardh 1824.

33. *Desmidium Swartzii* Ag.

(Figs. 85, 89)

Ralfs, *Brit. Desm.*, 1848, p. 61, Pl. 4; W. and G. S. West and N. Carter, *Mon. Brit. Desm.*, V, 1923, p. 246, Pl. 163, Figs. 5-8; Smith, *Wisconsin phytoplankton*, pt. II, 1924, p. 144, Pl. 88, Figs. 1-2; Krieger, *Die Desmidiaceen der Deutsch. Limn. Sunda Expedition*, 1932, p. 221, Pl. 26, Fig. 8.

Filaments spirally twisted, breadth of cells about twice the length, moderately constricted; apex of semi-cell broadly truncate,

with a short connecting process at each angle of the cell. Spaces between the cells faintly visible. Vertical view triangular with concave sides, angles broadly rounded. Chloroplasts massive, axile with broad projections running to the angles of the cells, projections incised about half the distance from the apex; pyrenoid one in each lobe, opposite to the sides.

Dimensions :

Length	13-15.6 μ
Breadth at centre	31-34 μ
Breadth at apex	27-29.9 μ
Isthmus	23-25.6 μ

Hab.—Kodaikanal Lake.

This form appears to be slightly narrower than West's form, but agrees with the measurements given by Krieger.

Genus *Gymnozyga* Ehrenberg, 1841

34. *Gymnozyga moniliformis* Ehrenberg

(Figs. 83, 88)

Turner, *Freshw. Alg. E. India*, 1893, p. 151, W. and G. S. West and N. Carter, *Mon. Brit. Desm.*, Vol. V, 1923, p. 255, Pl. 165, Figs. 8-9; Smith, *Wisconsin phytoplankton*, pt. II, 1924, p. 146, Pl. 87, Fig. 11; Brühl and Biswas, *Alg. of Loktak Lake*, 1926, p. 314, Pl. 15, Fig. 157; Krieger, *Die Desmidiaceen der Deutsch. Limn. Sunda Expedition*, 1932, p. 221, Pl. 26, Fig. 10.

Filaments with barrel-shaped cells; semi-cells with a small basal inflation and an extremely median constriction, lateral margins straight; apex broad and truncate. Vertical view circular with two opposite mamillæ. Chloroplasts axile, with a central big pyrenoid and about 6 radiating plates.

Dimensions :

Length	29 μ
Breadth at centre	17 μ
Breadth at the apex	11.9 μ

Hab.—Kodaikanal Lake.

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Statement showing the distribution of the forms recorded in this paper

Names of the forms	Place of collection in S. India	Previous places of collection in India	Author
<i>Gonatozygon Kinahani</i> (Arch.) Rabenh. ..	Kodaikanal	Assam, Burma, Bengal	Carter, W. and G. S.
<i>Netrium digitus</i> (Ehrb.) Itzigs and Rothe ..	do.	Ceylon, Burma, Hyderabad	Fritsch, W. and G. S. West, Carter, Turner
<i>Closterium libellula</i> Focke var. <i>putneyensis</i> var. nov. ..	do.
<i>Closterium Kutzingii</i> Breb. ..	do.	Ceylon, Burma, Bengal	Crow, W. and G. S. West, Turner
<i>Closterium Diana</i> Ehrenb. ..	do.	Burma, Ceylon, Bombay	Joshua, W. and G. S. West, Schmidle
<i>Closterium didymotocum</i> Corda var. <i>annulatum</i> var. nov. ..	do.
<i>Pleurotenium Trabecula</i> (Ehrenbg.) Nägeli ..	do.	Ceylon, Burma, Bengal	Fritsch, W. and G. S. West, Turner
<i>Pleurotenium minutum</i> Delp. var. <i>gracile</i> Wille ..	do.	Ceylon	W. and G. S. West
<i>Pleurotenium Kayei</i> Rabenh. ..	do.	Assam, Burma, Bengal	Carter, Joshua, Lagerheim
<i>Pleurotenium tessellatum</i> Joshua var. <i>bulbosum</i> Krieger. ..	do.
<i>Euastrum sinuosum</i> Lenorm ..	do.	Burma, Bengal	Carter, Joshua, Turner
<i>Micrasterias pinnatifida</i> (Kütz.) Ralfs. ..	do.	Ceylon, Loktak Lake, Bengal, Chittagong, Burma	W. and G. S. West, Brühl and B. was, Carter, Joshua, Turner

Statement showing the distribution of the forms recorded in this paper—(Contd.)

Names of the forms	Place of collection in S. India	Previous places of collection in India	Author
<i>Microsterias incisa</i> (Breb.) Ralfs. var. <i>Wallichiana</i> Turner	Kcdalkanal	Loktak Lake, Bengal, Assam, Burma	Brühl and Biswas, Carter, Joshua, Turner
<i>Cosmarium moniliforme</i> (Turp.) Ralfs. forma <i>punctata</i> Lagerh.	do.
<i>Cosmarium moniliforme</i> (Turp.) Ralfs. forma <i>panduriformis</i> Heimerl	do.
<i>Cosmarium obsoletum</i> (Hantzsch) Reinsch.	do.	Bengal, Ceylon, Burma	Lagerheim, Crow, W. and G. S. West, Joshua, Turner
<i>Cosmarium globosum</i> Buhl.	do.	Ceylon, Burma	W. and G. S. West, Joshua
<i>Cosmarium pachydermum</i> Lund. var. <i>indicum</i> var. nov.	do.
<i>Xanthidium sermanillatum</i> West and West var. <i>pulneyensis</i> var. nov.	do.
<i>Arthrodesmus subulatus</i> Kutz.	do.	Ceylon, Burma, Bengal	Crow, Joshua, Turner
<i>Staurastrum corniculatum</i> Lund. var. <i>spinigerum</i> West	do.
<i>Staurastrum Tohopetadigense</i> Wolle	do.
<i>Staurastrum unicolorne</i> Turner var. <i>gracile</i> var. nov.	do.

<i>Staurastrum conlectum</i> (Turner) var. <i>inevolutum</i> Turner	do.	Bengal	..	Turner	..
<i>Staurastrum longibrachiatum</i> (Borge.) Gutw. var. <i>intermedium</i> var. nov.	do.
<i>Staurastrum retusum</i> Turn. var. <i>punctulatum</i> Eichl. and Gutw.	do.	Ceylon, Burma	..	W. and G. S. West	..
<i>Staurastrum gladiosum</i> Turner
<i>Staurastrum furectum</i> (Ehr.) Breb.
<i>Staurastrum hexacerum</i> (Ehr.) Witttr.	..	Burma, Bengal	..	W. and G. S. West, Turner	..
<i>Staurastrum columbetoideum</i> West and West	..	Ceylon	..	W. and G. S. West	..
<i>Staurastrum arachne</i> Ralfs. var. <i>pulneyensis</i> var. nov.	do.
<i>Staurastrum gracile</i> Breb. forma
<i>Hydrotheca dissiliens</i> (Sm.) Breb.	..	Ceylon, Loktak Lake, Bengal, Burma	..	Pritsch, W. and G. S. West, Brühl and Biswas, Wallich, West and West, Turner	..
<i>Desmidiium Suarizii</i> Ag.	..	Loktak Lake, Bengal	..	Brühl and Biswas, Wallich, Turner	..
<i>Gymnozyga moniliformis</i> Ehrenb.	..	Ceylon, Loktak Lake, Bengal, Burma	..	Pritsch, W. and G. S. West, Brühl and Biswas, Wallich, W. and G. S. West, Turner	..

A NOTE ON HETEROTHRICHOPSIS VIRIDIS gen. et sp. nov.

BY M. O. P. IYENGAR, M.A., PH.D. (LOND.), F.L.S.

AND

MISS S. KANTHAMMA, B.Sc. (HONS.), M.Sc. (MADRAS)

University Botany Laboratory, Madras

Received for publication on January 20th, 1941

THE authors published in November 1940 an account of a new algal genus under the name of *Ulothrichopsis* (M. O. P. Iyengar and S. Kanthamma, "A Note on *Ulothrichopsis viridis* gen. et sp. nov.", *Journ. Ind. Bot. Soc.*, 1940, 19, Nos. 1-3, 167-70). Since the publication of this paper, the authors find that the name *Ulothrichopsis** has been used for another new genus of the Ulotrichaceæ by Wichmann in 1937 (L. Wichmann, "Studien über die durch H-Stück-Bau der Membran ausgezeichneten Gattungen Microspora Binuclearia, Ulotrichopsis and Tribonema," *Pflanzenforschung*, 1937, Hft. 20, 81-83). The authors, therefore, rename their new genus as *Heterothrichopsis* gen. nov.

DESCRIPTION

Heterothrichopsis gen. nov. (= *Ulothrichopsis* Iyengar and Kanthamma, non *Ulothrichopsis* Wichmann).

Thallus filamentous and unbranched, consisting of a few cells only placed in a row; each cell containing a single nucleus and one or more parietal plate-like chloroplasts with one or more pyrenoids in each. Vegetative reproduction by fragmentation of the filaments into shorter lengths consisting of one or more cells. Asexual reproduction by aplanospores. Zoospores or gametes unknown.

Heterothrichopsis viridis sp. nov. (= *Ulothrichopsis viridis* Iyengar and Kanthamma).

General characters same as those of the genus. Filaments one to four cells placed in a row; cells $6.2-7.9\ \mu$ broad and $15.8-33.3\ \mu$ long. Chloroplasts 1-8 (usually 2-4) in each cell.

Hab.—In a laboratory culture of soil algæ from Tambaram near Madras.

*The name is spelt by Wichmann without an "h" after the "t".

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[No. 3

TUBERCULINA ON *UROMYCES HOBSONI* VIZE

BY M. J. THIRUMALACHAR

Department of Botany, Central College, Bangalore

(Communicated by Dr. M. A. Sampathkumaran)

Received for publication on September 29, 1940

As far back as 1880, the genus *Tuberculina* was first described by Saccardo⁴ as a parasite on the pine rust *Cronartium ribicola*. Rostrup³ further studied the form *Tuberculina maxima* Rostrup (*T. persicina*), and reported its occurrence in Denmark and Germany. Tubeuf^{7,8} was the first to study the cytological and cultural aspects of this fungus. Experiments were carried out to utilise this fungus as a means of controlling the devastating effects of *Cronartium ribicola* on *Pinus strobus*. Similar experiments were taken up in England, Denmark and Belgium, and the work of Spaulding⁵ and Rohmeder² showed that the development of aeciospores was prevented to a certain extent in *Cronartium ribicola* by the parasitism of *Tuberculina maxima* Rostrup.

Widespread efforts were made to find out the occurrence of this fungus in the pine growing areas of America, and various workers such as Hubert¹ and others recorded this on species of *Cronartium*, including *C. ribicola* and *C. coleosporoides*. In India there is no record of this fungus on pines. The only record is by Sydow and Mitter⁶ who found a species parasitic on the uredosori of the rust *Phakopsora cronartiiformis* (Barclay) Diet. on *Vitis himalayana*, and named the fungus *T. costaricana* Syd.

In studying the life-history of the rust fungus *Uromyces Hobsoni* Vize. on *Jasminum grandiflorum*, the writer observed this interesting fungus on the sorus forms. Hypertrophied flower buds of *Jasminum grandiflorum* which had developed aecia and pycnia, were covered with a purple black coating of *Tuberculina*. In places where the infection was sparse, the conidiosori could be mistaken macroscopically for the telia of *Uromyces Hobsoni* Vize. It is quite well known that in *Uromyces Hobsoni* Vize. the telia pycnia, and aecia are associated with one another on the same host.⁹

The material was collected and killed in fixing fluids as Flemming's and Allen's Fluids for sectioning after embedding in paraffin. Heidenhain's Iron-alum Hæmatoxylin with Orange G was used for staining.

DESCRIPTION OF THE FUNGUS

The genus *Tuberculina* established by Saccardo in 1880,⁴ include those urediniculous fungi imperfecti which have hyaline conidia and hyaline hyphæ with smooth or nearly smooth sporodochia. Only the conidiospore stage is known. In microtome sections, the infection of aecia and pycnia of *Uromyces Hobsoni* was studied in detail. The mycelia form knots in the intercellular spaces, right on the side of the pycnium. The gradual disintegration of the pycnium on the contiguous sides takes place. In Fig. 1 the pycnium is attacked on either side, and a remnant of it is remaining over in the centre. In later stages the pycnia are completely surrounded by the mycelium, which extensively develops sporodochia (Fig. 2).

In numerous cases the infection of aecia was also observed. The hyphæ enter the aecial cup, and attack the spore mother-cells, spores, peridial cells, and even the basal cells (Fig. 3). The conidia are acrogenous and not pleurogenous. The conidiospores are abstricted at the tip exogenously, and are not catenate. Two neighbouring groups of conidiosori may coalesce and form a continuous layer. The mycelia are binucleate. The conidiophores are binucleate (Fig. 5a), and the spores developed at the tip are spherical, thin walled and binucleate (Fig. 5b). The spores measure $8.6 \mu \times 6.8 \mu$.

While germination studies were not carried out to obtain infection on *Uromyces Hobsoni* Vize., it was interesting to note that some of the conidiospores lying scattered near the conidiophores had produced small germ tubes. This ready germination of the spores soon after being abstricted off from the conidiospores, might facilitate infection of the rust fungus effectively.

Hubert¹ in his description of *Tuberculina maxima* Rostrup on *cronartium ribicola* states, that the mycelia of the hyperparasite attacks mostly spores of aecia and pycnia and not the mycelia in the host tissue. However he mentions the attack of the pycnial stroma under the epidermis in some cases. In *Tuberculina* attacking *Uromyces Hobsoni* Vize. the infection of aecial plectenchyma and even pycnial mycelium was observed. The entire disintegration of the aecial plectenchyma is followed by the development of sporodochia, which ruptures through the epidermis (Fig. 4). The spores are dispersed by the wind.

The keen interest that has been for long evinced in the life cycle of this fungus by the research workers in Europe and America is explained by the fact, that it may possibly afford a means of controlling the blister rust of pines. Tubeuf^{2,3} and Hubert¹ report

partial successes. Decrease in the production of aeciospores has been confirmed by various workers. In *Uromyces Hobsoni* Vize., the normal development of telia always takes place within the aecial cup and rarely in pycnia. In infected aecia telial development was found to be completely suppressed, thereby preventing the development of the resting spore form which is the only means of carrying on the infection in the next season.

Dr. B. B. Mundkur is of opinion that this species of *Tuberculina* on *Uromyces Hobsoni*, comes nearest to *Tuberculina costaricana* Syd. on *Phakopsora Cronartiiformis* (Barclay) Diet. recorded for India by Mundkur (Fungi of India Supplement I, Scientific Monograph Imperial Council of Agric. Res., 12, p. 37, 1938) and that it resembles, in certain respects *Tuberculina pelargonii* Patoulliard parasitising the aecia of *Puccinia granularis* on *Pelargonium* Sp. in Arabia. In the absence of Sydow's or Patoulliard's type specimens for comparison, the *Tuberculina* species on *Uromyces Hobsoni*, Dr. Mundkur thinks, may tentatively be referred to *Tuberculina costaricana* Syd.

In conclusion the writer wishes to acknowledge his indebtedness to Dr. B. B. Mundkur, Imperial Agricultural Research Institute, New Delhi, for his valuable help in identifying the species and helpful suggestions, and to Dr. M. A. Sampathkumaran, M.A., Ph.D., S.M. Professor of Botany, Central College, Bangalore, for guidance and encouragement during the course of this work.

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EXPLANATION OF PLATE I

- FIG. 1. Photomicrograph of an infected pycnium of *Uromyces Hobsoni*, showing the development of conidiophores on either side. Disintegration of pycnosporophores can be made out. $\times 50$.
- FIG. 2. Photomicrograph showing the sporodochium completely enveloping two pycnia 'Py'. $\times 50$.
- FIG. 3. Camera lucida drawing of an infected aecia showing the conidiophores at the surface. The extension of hyphae between the chains of aeciospores down the aecial cup can be made out. $\times 160$.
- FIG. 4. Camera lucida drawing, showing the infection of plectenchyma of aecia, and the development of conidiophores below the epidermis. $\times 200$.
- FIG. 5a. Conidiophores showing the development of spores at the tip exogenously. $\times 450$.
- FIG. 5b. Conidiospores showing binucleate condition. $\times 450$.

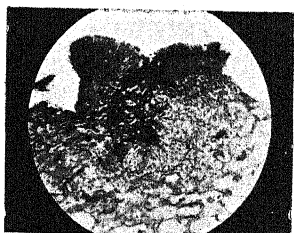


FIG. 1

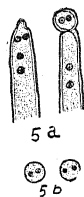


FIG. 5

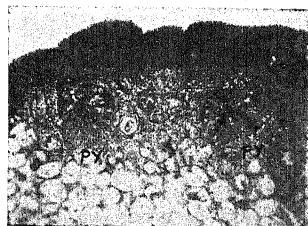


FIG. 2

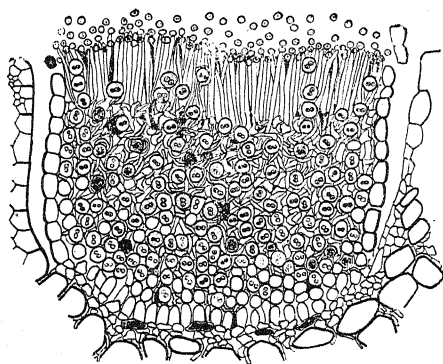


FIG. 3

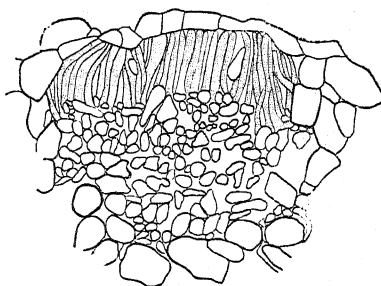


FIG. 4

M. J. THIRUMALACHAR

TUBERCULINA ON UROMYCES HOBSONI VIZE



CHROMOSOMAL ALTERATIONS INDUCED BY X-RAYS IN BAJRI (*Pennisetum* *typhoides* STAPF & HUBBARD)

BY N. KRISHNASWAMI, B.Sc., Ph.D. (KIEL.)

AND

G. N. RANGASWAMI AYYANGAR, F.N.I., I.A.S.

Millets Breeding Station

Agricultural Research Institute, Coimbatore

Received for publication on October 22, 1940

KUMAR AND JOSHI (1939) using a metalax, water cooled, tungsten target tube, exposed to X-rays in *Pennisetum typhoidium*, ovaries and anthers (45 to 60 k.v. ; 5 ma. 5 mins. ; at 12 and 14 inches) and seeds soaked in water for 24, 48 and 72 hours (50 k.v. ; 5 ma. ; 10-15 mins. ; 12 inches). They obtained no "significant mutations" under the given dosages, but however observed that mature plants were shorter in height, had lesser number of tillers, but gave longer earheads and the setting on the average was not good.

Resting seeds of Bajri were treated with X-rays in this Station and some of the results are given below :

MATERIAL AND METHODS

Resting dry seeds of P.T. 700 'Whip' Cumbu from Nigeria were used in this experiment. The seeds were treated at the Physics Department of the Indian Institute of Science, Bangalore. Our thanks are due to Sir C. V. Raman and his assistant Dr. P. Neelakantan for the facilities and help rendered.

The seed samples were put into cellophane bags and spread single layered to an area of about 4 cm. \times 2 cm. The four limits were marked off by phosphorescent paper strips. The bags were fastened by paper-clips to cardboard strips and the latter fixed by clamps to stands and raised to the level of the windows.

Specification of the X-Ray Tube.—A water cooled Spektroanalyt Röntgen Apparat, manufactured by Richard Scifert & Co., Hamburg, provided with Lindeman-glass windows, $\frac{1}{2}$ wave rectification and copper anticathode was used and operated at 15 ma. 42,000 K.V.P. and a target distance of 19 cm. The seeds were exposed for 1, 2, 3 hours duration.

The exposed seeds were sown in pots at the Millets Breeding Station and later transplanted into the field. The germination in all the three treatments were good but the survivors were few. Many died even before the coleoptile could emerge out properly. Some albinos were also noted.

Treatment	Duration	No. of seeds treated	Greens	Albinos	% Survivors
A	1 hour	176	82	37	46.5
B	2 „	200	6	47	3
C	3 „	142	nil
Control		200	192	..	90

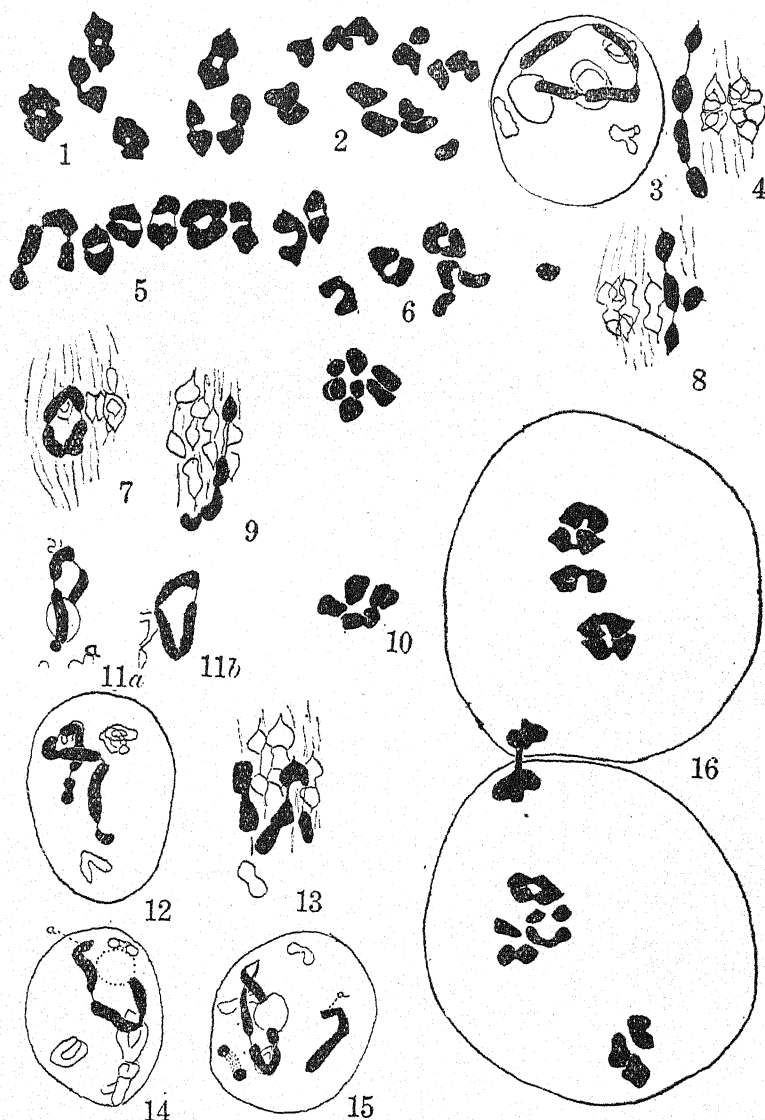
In the three hours exposure, especially, the seedlings were seen to break through the soil surface but failed to develop further and all died. Thus 3 hours proved lethal and 2 hours sub-lethal under the given dosages. All the albinos died.

The mature plants were all examined for any chromosomal mutants that might have occurred. Four plants from treatment A showed aberrations and their behaviour is given below :

Method.—Only the meiotic phase was examined. The flower-buds were fixed in Acetic-alcohol (1:2 mixture). The cursory examinations were done in temporary aceto-carmin smears. Paraffin sections of the required material were cut. The sections were stained according to Feulgen's technique and counter-stained with Fast-green. All figures are drawn to a magnification of about 3,000, while those from the acetocarmine mounts have been drawn to a magnification of about 2,300. Figures reduced to $\frac{1}{4}$ in reproduction.

The acetocarmine smears showed the cells and chromosomes swelled to almost twice the size of those in the permanent preparations. Hence the figures drawn from the smears appear larger than those from paraffin preparations. Meiotic stages from diakinesis onwards only have been observed.

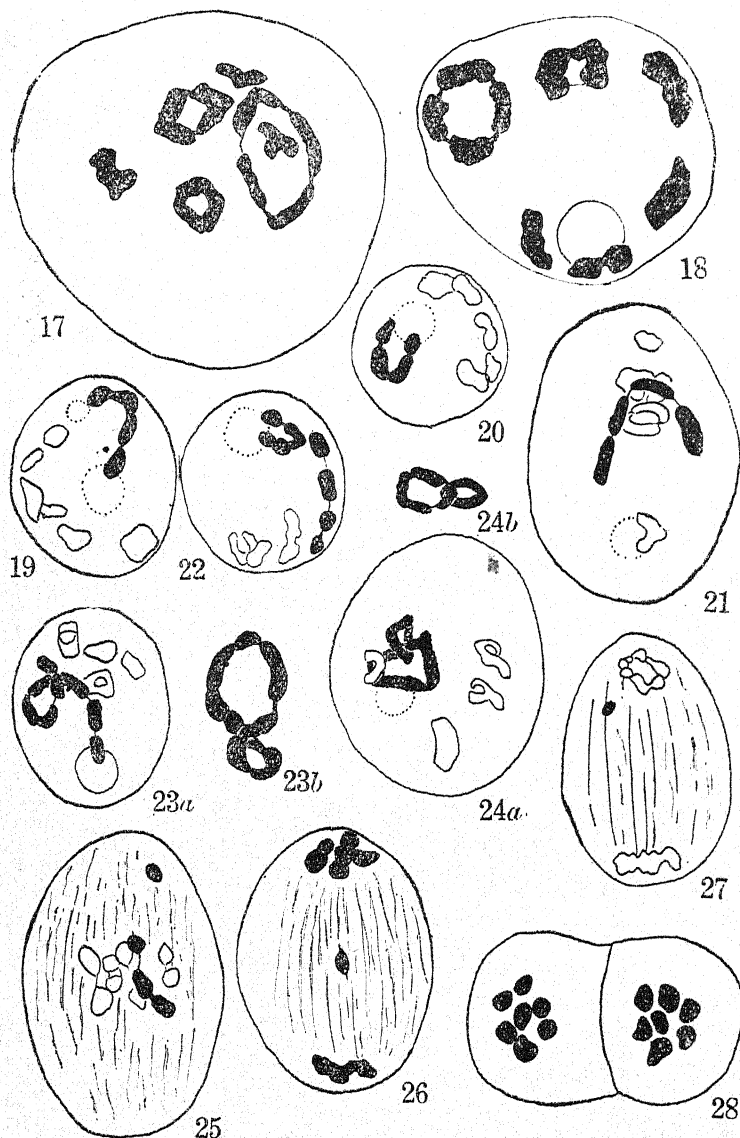
(Plants Nos. 53, 60 and 64).—Normally *Pennisetum* forms four ring bivalents and three rod bivalents. The anaphase shows regular separation of 7 univalents to each pole (Fig. 1, M-I chromosomes spaced out 2, A-I). But in these treated plants there occurred instead of seven bivalents 5 bivalents and one ring of four, or a chain of four and 5 bivalents (Fig. 3, a ring of 4 in plant 64; Fig. 4, a chain of four M.I. in plant 53; Fig. 7, ring of 4 at M.I. in plant 60). The ring usually appears as a chain at metaphase I due to dissolution of chiasma (Fig. 5). In some cases a chain of three and a univalent may appear (Figs. 6 and 8). At anaphase the associated chromosomes are distributed commonly 3 to 1 pole and 1 to the other (Fig. 9), so that there occur 8 and 6 at the poles (Fig. 10) at telophase.



Figs. 1-16. Meiosis in plants 53, 60 and 64. Explanation in text (Figs. 1, 2, 5, 6, 10, 13 and 16 acetocarmine smear). Plant 53—Figs. 4, 8, 9, 12 and 13; Plant 60—Fig. 7; Plant 64—Figs. 2, 3, 5, 6, 10, 11a and b, 14, 15 and 16 (Cytomixis).

Some abnormalities were also noted. Rings of three or a chain of three with a short fourth segment were observed (Figs. 11b and a). A chain of 6 chromosomes is shown in Fig. 12. Fig. 13 is an anaphase in which instead of the normal end chromosome (as in Fig. 9)

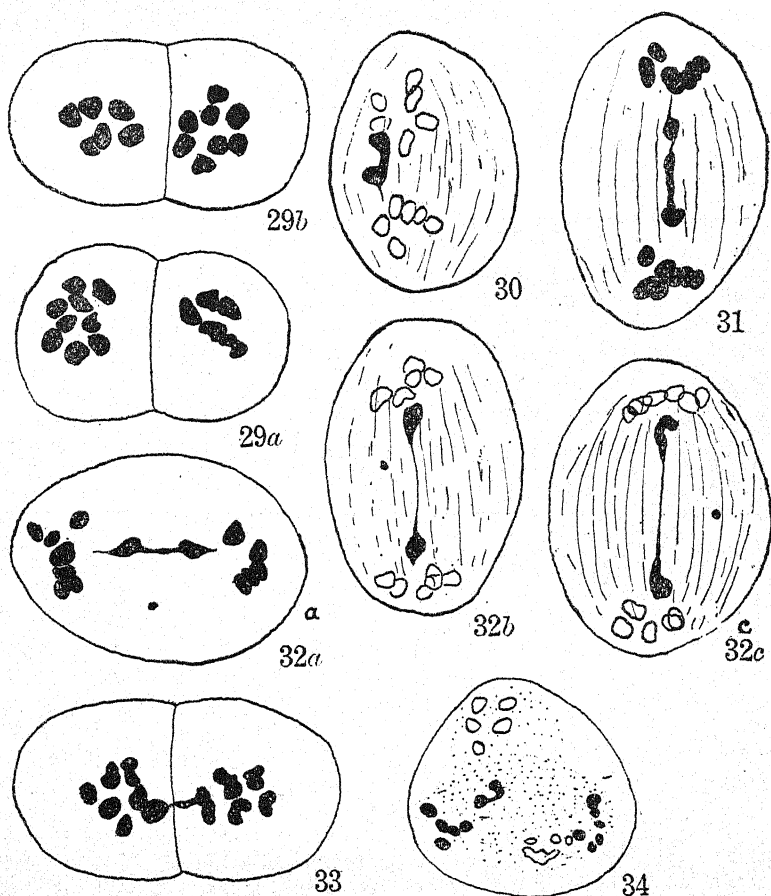
one of the middle is pulled to one pole while the other three to the opposite pole. In a few cases translocation of a short segment appears to have occurred (Figs. 14 and 15 the piece marked *a*). Fig. 16 is at M-I, one bivalent has got into the neighbouring cell probably due to cytotoxicity.



Figs. 17-28. Meiosis in plant 56 (Figs. 17, 18 and 23*b* acetocarmine smear). Explanation in text.

The plants were semisterile.

Plant 56.—This plant also showed rings of four (Figs. 17 and 18) or chains of four (Figs. 19–21). The nucleolar chromosomes seem to have been involved in this plant. Fig. 19 shows both the end chromosomes of the chain attached each to one nucleolus and Fig. 20 with both attached to the same nucleolus. In some of the pollen mother-cells two rings of four or a chain of four and a ring were observed. In two cases the two rings had associated into single chain of 8 (Figs. 23*a*, open chain; *b*, closed chain). Interlocking occurred in some cases (Figs. 24*a* and *b*). At anaphase as in the case of the previous three plants the associated chromosomes separated into 3 and 1 (Fig. 25). In some of the P.M.C.'s a univalent was seen on the spindle towards late anaphase (Fig. 26) or at telophase at one of the poles (Fig. 27). It is probable that one of the chromosomes of the chain separates early and two of the others



Figs. 29–34. Meiosis in plant 56. Explanation in text.

go to one pole leaving the middle one at the plate. In Fig. 25 the connection between the top one and the lower two at the equatorial plate appear to be stretched and in tension so that the lower two separate themselves from the top one. At the anaphase-II sometimes a univalent was seen dividing (Fig. 34). The distribution of the chromosomes in normal cells is 7-7 (Fig. 28). The cells with rings may show 8-6 or occasionally 9-5 showing that the ring had failed to disjoin and migrated as such to one pole.

Fig. 30 shows a delayed disjunction. Chromatin bridge and a small fragment are formed at Anaphase-I very commonly (Figs. 31 and 32). The bridges occur also in cells without chains or rings indicating that inversion has taken place in one of the chromosomes other than those with segmental interchange. The ring was not observed to form the bridge. Fig. 33 shows a normal 7-7 distribution with remnant of first division bridge. Tetrads are normally formed but the plant is semisterile.

The following gives the frequency of cells with segmental interchange :—

Plant No.	Cells without rings	Cells with ring or chain	% of cells with rings
53	332	71	17.6
56	126	85	40.2
60	70	30	30.0
64	66	45	40.5
TOTAL	.. 594	231	28.0%

That the association of non-homologous chromosomes in rings or chains is due to structural hybridity involving segmental interchange is well established. Such types are classed as Interchange heterozygotes (Darlington, 1937). Similar cases have been recorded as occurring as a result of irradiation (Sansome and Philp, 1939) and also in nature. Amongst the cereals they have been noted in *Zea mays* (Stadler, 1931, McClintock, 1930), *Oryza sativa* (Ramiah *et al.*, 1934), *Avena fatua* (Huskins, 1925), *Eleusine coracana* (Krishnaswamy, 1939), *Secale cereale* (Darlington, 1937), *Triticum monococcum* (Katayama, 1935, 1935a). The occurrence of chains of three is obviously owing to either early dissolution of the chiasma or failure of chiasma (Darlington, *l.c.*). The genetical implications of the segregation of the interchanged chromosomes have been fully discussed by Sansome and Philps (*l.c.*). Selfed seeds from these four plants have been gathered with a view to study the progeny.

SUMMARY

1. The dry seeds of *Pennisetum typhoides* S. and H. have been treated with X-rays for 1, 2 and 3 hours durations. The two hours exposure proves sub-lethal and the three lethal.

2. In the progeny of the exposed seeds four plants with segmental interchange were noted. The cytological behaviour of these plants is described.

3. One of the plants, in addition to the segmental interchange, showed inversion in one of the chromosomes not involved in the interchange.

4. The plants were all semisterile. The selfed seeds of these plants have been gathered for further study.

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ANOMALOUS STRUCTURE OF THE STEM
OF NYCTANTHES ARBORTRISTIS L.

BY GIRIJA P. MAJUMDAR

Botanical Laboratories, Presidency College, Calcutta

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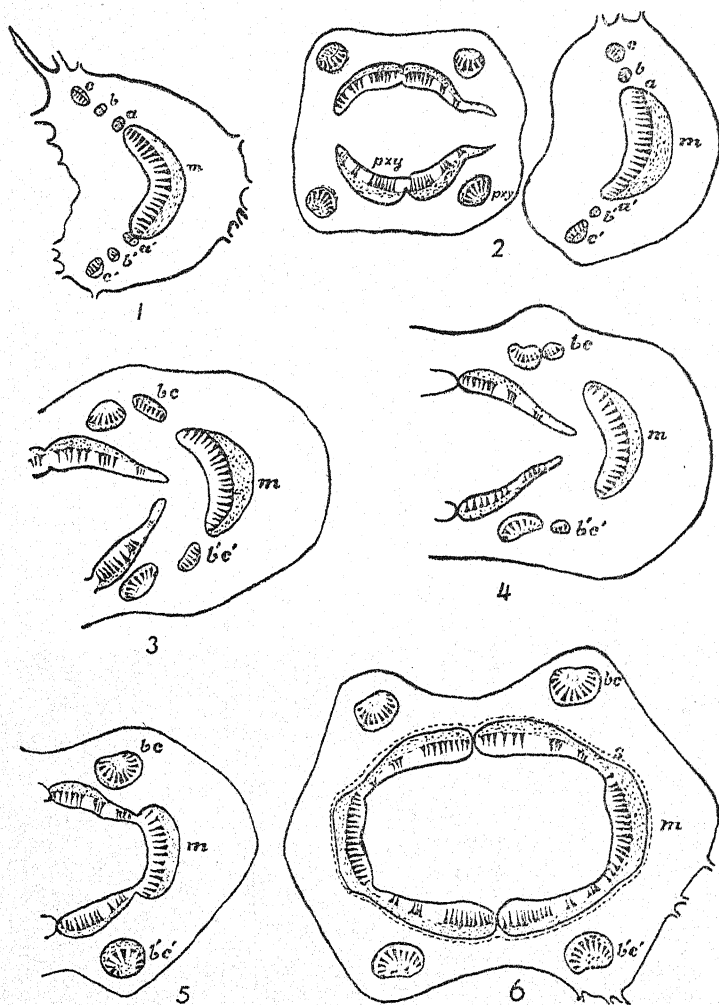
At the Twenty-third Session of the Indian Science Congress⁵ I read a short paper before the Botany Section on the structure of *Nyctanthes* stem, and thought of working out its details in future. Meanwhile Mr. Fotidar³ has published an account of the primary vascular system of this plant. As Mr. Fotidar's account appears to me to be rather incomplete in some respects, particularly in regard to the occurrence of the band of fibrous pericycle (p. 43 and fig. 1), this note, which I prepared while working in the Botanical Laboratories of the Leeds University, may be taken as a supplement to Mr. Fotidar's paper.

Nyctanthes arborescens L. is a member of the Oleaceæ, and is a woody shrub cultivated in gardens and also found wild in Chittagong. Solereder⁶ states in relation to the family Oleaceæ, "the axis shows no anomalous structure, even in the climbing species of *Jasminum*" (p. 525). Examination of the plant does show, however, anomaly in the presence of cortical bundles with inverse orientation of their elements.

The plant has a decussate phyllotaxis. The leaf-trace is composed of three bundles, but this is associated with only one leaf-gap, since lateral bundles from the leaf run down the internode below as cortical bundles, and are not associated with gaps in the vascular ring of the axis.

The trace system of the vascular ring is normal; the pair of bundles contributed by a pair of leaves run down vertically until they fork around the leaf-gap of the lower next pair on the same orthostichy, the pair from leaves inserted at the other two orthostichies behaving in a similar manner.

In their course through the internode below the leaf insertion, the main trace bundles join the vascular ring of the axis, but the regions containing the vessels indicate the position of the main bundles, the traces of the axillary bud and downward prolongations of forked bundles from higher pairs of leaves. The tissue around the leaf-gaps are evidently slow to repair since fairly down the internode the main bundles from the pair of leaves at the next higher node but one are already showing commencement of forking around the leaf-gap (fig. 6).

Figs. 1-6. *Nyctanthes arbortristis* L.

Figs. 1-6. Anomalous stem structure of *Nyctanthes arbortristis* L. A series of transverse sections of the stem just above, through and below a node. Fig. 1 shows the structure of the petiole; fig. 2 that of internode and leaf-base just above the node; fig. 3 the same at the insertion of the leaf; figs. 4 and 5 at the node; and fig. 6 a little below the node; *m*, main trace bundle; *a*, *b*, *c* and *a'*, *b'*, *c'*, laterals; *S*, starch sheath; and *pxy*, protoxylem. All figures are drawn under a micro-projection apparatus, semi-diagrammatic ($\times 20$).

Cortical bundles occur in a number of plants (De Bary,¹ p. 256 f.; Solereder,⁶ p. 1159—under anomalous structure of the axis). Solereder⁶ and Prillieux⁶ who worked on *Nyctanthes* apparently

failed to record the presence of cortical bundles in this plant. The anomaly was perhaps for the first time reported by me in 1936.

The type of anomaly noticed in this plant strangely enough corresponds in the main outline to what has been described for the widely separated genus *Calycanthus* of the Calycanthaceæ. Thus the cortical bundles of *Nyctanthes* like those in *Calycanthus* are peculiar in building up an entirely cortical system which is never directly connected with the main axial ring, and also in the way in which the marginal bundles which run in the petiole with normal orientation of xylem and phloem swing round at the node of insertion so as to show inverse orientation in their courses in the axis.

Mr. Fotidar³ has described (p. 43) and figured (fig. 1, p. 44) the presence of a sclerenchymatous pericycle as a notable feature in *Nyctanthes*, and according to him Solereder⁶ regards this feature as characteristic of the family Oleaceæ (p. 43). But Solereder (p. 525) mentions *Jasminum*, *Syringa* and others as having isolated groups of bast fibres in the place of a continuous fibrous pericycle, and quotes Pitard (1901) who found the pericycle to contain isolated groups of bast fibres in *Fontanesia* sp. and *Forsythia* sp. (p. 982).

In my material, collected from a garden in Calcutta, the comparative study of the stages of tissue differentiation in the axis gives a different picture. The starch sheath differentiates very early and persists for a long time after the secondary growth has started. Between the starch sheath and the vascular ring a layer or two of pericycle may be distinguished at an early stage of tissue differentiation, but soon after fibre mother cells in groups are seen to organise at the outer periphery of the phloem ring. In fairly old axes these groups are differentiated into isolated patches of lustrous white unligified fibre cells, and form a discontinuous ring of sclerenchyma of variable depth around the vascular ring. At some places the fibrous cells abut directly on the endodermis by crushing and obliterating the intervening cells of the pericycle; at other places, particularly between adjacent groups, a few of the original cells of the pericycle may still be distinguished. Identical fibre cells singly or in groups of two, rarely three, are also secondarily differentiated just at the outer periphery of the phloem of the cortical bundles. Developmental studies confirm that these isolated groups of fibre cells belong to the phloem and not to the pericycle. In origin and nature they are like phloem fibres of jute, hemp, potato, tobacco and similar other plants (Kundu⁴).

In conclusion it may be pointed out that the statement often made in Solereder and other treatises that the "pericycle contains isolated groups of bast fibres" is misleading and should not be taken literally, since bast fibres, as later investigators have shown, belong to the phloem, and pericycle, when present, is altogether a different tissue region outside the phloem. Eames and MacDaniels² state that 'bast fibres' should be discontinued as a technical term (p. 75).

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A MORPHOLOGICAL STUDY OF THE FLOWER
OF *BLYXA ECHINOSPERMA* HOOK. F.

BY K. RANGASAMY

Department of Botany, Central College, Bangalore

(Communicated by M. A. Sampathkumaran)

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INTRODUCTION

THE genus *Blyxa* belongs to the family Hydrocharitaceæ and order Helobiales. Many genera of this family have already been investigated by different workers, *Elodea* (Wylie, 1904), *Ottelia* (Narasimhamurthy, 1935), *Vallisneria* (Rangasamy, 1934; Witmer, 1937), *Enalus* (Kausik, 1940), *Hydrocharis* and *Stratiotes* (Clausen, 1927). The present study of *Blyxa echinosperma* Hook. f. would complete the investigation of all the genera of the family with the exception of *Lagarosiphon*, *Boottia*, and *Halophila*.

MATERIAL AND METHODS

The genus *Blyxa* has many species distributed in Indian inland waters, some bearing unisexual and others hermaphrodite flowers. The dioecious species are more numerous than the bisexual types. *Blyxa echinosperma* is a hermaphrodite species commonly distributed in South India. It is easily identified by its echinate and two tailed seeds.

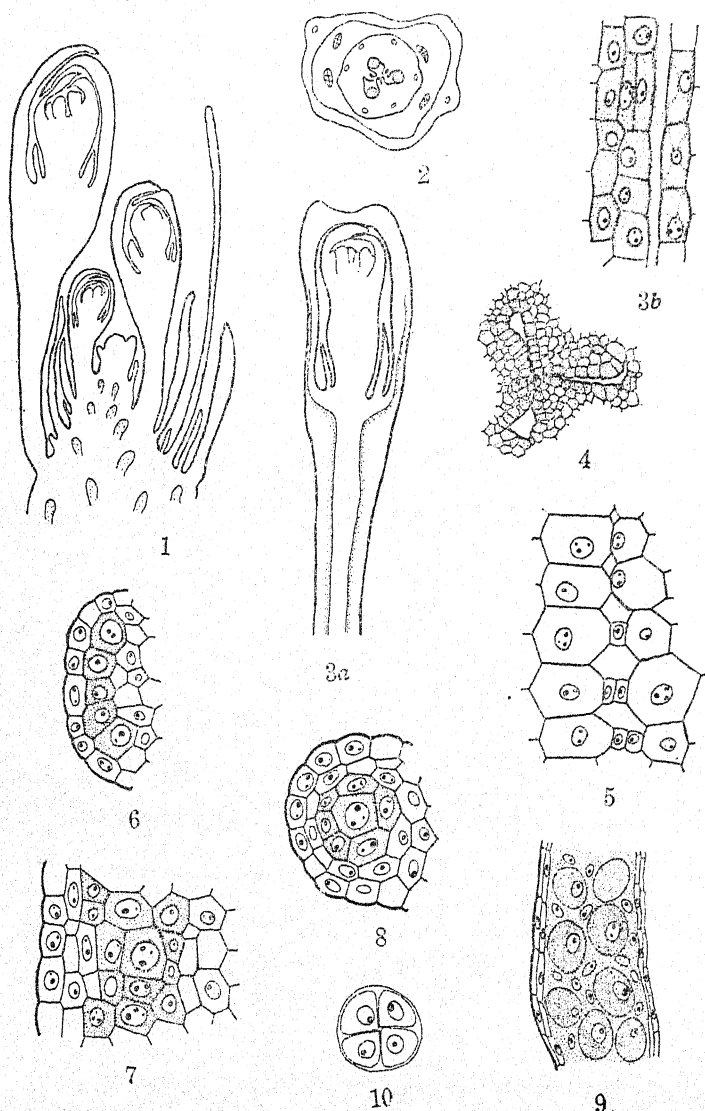
The material was collected at Talaguppa, a village near Jog Falls in Mysore State, from a stagnant pool. The plant very much resembles *Vallisneria spiralis* in its appearance, but lacks the coiled peduncle of the female flower and the short male inflorescence of *Vallisneria*. The hermaphrodite flowers bloom just above the level of water and there is no withdrawal after pollination. The pollination is effected either by wind or insects. The length of the flower stalk depends upon the depth of the surrounding water as in *Vallisneria*. These do not grow beyond a depth of five or six feet, being confined only to the margins of deep stagnant pools in the Western Ghats.

The material was fixed in Nawaschin's fluid in the field and was later dehydrated and cleared in grades of alcohol and xylol. Since each cluster contained a number of flowers at different stages of development, it was easy to study the growth and differentiation of the various parts. Each flower cluster is covered over by a spathe-like sheath, with plenty of mucilage. This was removed and the cluster was put into the fixing fluid. The wall of the ovary at the fertilization stage and after develops lot of cutin, which hinders infiltration by paraffin. Therefore, the fertilised ovules and the seeds containing various stages of embryo were removed

from the ovary and fixed separately. The material was cut at a varying thickness of 10 and 14μ and stained with iron-alum hæmatoxylin.

THE FLOWER

The flower cluster shows a number of flowers arising in acropetal succession along with numerous bract-like scales and mucilage hairs (Fig. 1). Some of the scales are empty while others contain



Text-figs. 1-10. *Blyxa echinosperma*.—Fig. 1. Long section of the inflorescence showing four flowers, bracts, and mucilage scales. Dotted

in their axils the developing flowers. In a longitudinal section, the axis of the inflorescence shows a number of cavities alternately arranged as shown in the figure. Each cavity is surrounded by mucilage secreting cells. The vascular bundles are not distinct at first, and appear only after the flowers have considerably advanced in their growth. At a certain height in the flower stalk, the flower spathe arises as a circular growth and its tip shows three projections which imbricate the flower at the tip. The youngest flower in (Fig. 1) shows the spathe primordium emerging out. The perianth lobes are three in number appearing higher up in the flower. There are three stamens alternating with these perianth leaves and arising on the top of the inferior ovary. The mucilage secreting hairs are found in the axil of the flower and the spathe. The cross section of the flower (Fig. 2), shows four or five of these hairs, while the longitudinal section shows two on either side. Each of these hairs consists of densely staining cells in the earlier stages, but later exhibits complete depletion of cell contents becoming brownish in appearance. The secreted mucilage also appears brown in section of young flowers. The significance of these mucilage hairs in the tender parts of the aquatics as a protection against harmful osmotic variations has been recognized by Goebel (1889-93). The presence of similar hairs in flowers has been noted in *Enalus* (Kausik, 1940) and in *Elodia* (Wylie, 1904).

Each flower when young shows two mucilaginous ducts along the length of the stalk on either side (Fig. 3a), which enter into the spathe. The ducts are narrow and develop schizogenously. Towards the outside, the cells appear to be rich in contents and take part in secreting mucilage (Fig. 3b), while on the inner side the cells are poor in contents. These lateral ducts later fuse at the base of the ovary. As the flower grows, the ducts grow wider and become irregular by the disorganisation of cells around them, but the mucilage system is fully formed even before the first appearance of the vascular traces in the flower.

The ovary differentiates rather late. When the microspore-mother cells are formed, three radiating grooves arise in the solid core of tissue beneath the perianth and the stamens. The cells bordering these canals become richer in contents (Fig. 4), as the

spaces indicate mucilage areas ($\times 80$). Fig. 2. Cross section of flower (ovary portion) with outer spathe, mucilage scales, ovary with three split placental ridges, ovules and six vascular groups ($\times 80$). Fig. 3a. Long section of flower with outer spathe, two mucilage scales, below, perianth lobes above and primordia of anthers. The mucilage ducts could be followed from the base of the flower ($\times 80$). Fig. 3b. Shows the schizogenous origin of mucilage duct with cells rich on one side ($\times 800$). Fig. 4. Cross section of young ovary having three slits with cells rich on sides ($\times 800$). Fig. 5. The nature of origin of aerenchyma in the wall of ovary and flower stalk ($\times 800$). Fig. 6. The hypodermal male archesporium ($\times 800$). Figs. 7 and 8. Long and Cross sections of anther locules to show a single layer of pollen mother cells, tapetum, parietal layer, and the epidermis ($\times 800$). Fig. 9. Old anther locule with pollen and periplasmodium ($\times 400$). Fig. 10. Pollen tetrad, isobilateral ($\times 800$).

nucleus in the microspore-mother cell prepares for meiotic divisions. The projecting ridges get gradually split up at the tip marked by rich placental cells.

The development of aerenchyma in the wall of the ovary and the flower stalk was followed and has been represented in (Fig. 5). The originally compactly arranged cells begin to show spaces at their corners. These spaces get enlarged by the formation of smaller cells between the larger ones. The formation of the smaller cell is preceded by the migration of the nucleus of the bigger cell to one side. After nuclear division a wall is laid down and thus a small cell is cut off. Such small cells have meristematic activity and by division form a chain of cells. The length of this chain determines the dimension of the air spaces. Such air spaces begin to form by the time the initials of the ovules are established.

MICROSPOROGENESIS

The primordia of the anthers arise very early in the history of the flower. Each primordium shows the formation of four lobes. A vertical section of one of these lobes discloses the formation of the archesporium in the hypodermal position (Fig. 6). This archesporial plate of five or six cells in length and one cell in width soon cuts off a layer of parietal cells. These parietal cells undergo further division to form the tapetum, which consists of cells that are as rich in contents as the sporogenous cells themselves (Fig. 7). In *Vallisneria* (Rangasamy, 1934; Witmer, 1937) and *Ottelia* (Narasimhamurthy, 1935) also, the parietal cell undergoes division to form both the wall layer and the tapetum. *Ottelia* has two wall layers while *Vallisneria* and *Blyxa* have only one.

The sporogenous cells have a limited number of divisions. There is only one, sometimes two layers (Figs. 7 and 8), of microspore-mother cells surrounded by tapetum. Thus the pollen grains are very limited in each anther.

The tapetal cells which are uni-nucleate very soon break down and the nuclei with the cytoplasm migrate into the mass of microspore-mother cells forming a persistent periplasmodium (Fig. 9), which feeds the male cells right from the tetrad stage. This kind of a plasmodium is a feature of common occurrence in Helobiales.

The tetrads show the pollen grains arranged in a bi-lateral manner (Fig. 10). In *Vallisneria*, Witmer (1937) records the same character, while in *Ottelia*, Narasimhamurthy (1935) observed in addition to the bi-lateral type of tetrads the linear and the tetrahedral types also. With the liberation of the microspore from the mother cell wall, the differentiation of the exine and intine takes place. The exine forms projections which become conspicuous later, making the pollen spinescent.

The mature pollen shows the presence of a tube nucleus and two sperm cells. The two sperm cells are placed back to back (Fig. 11), with their projecting ends pointing outward. The two

cells remain close together only when they are formed. Soon they get loose from each other and lie freely in the pollen grain. The wall of the sperm cells in mature pollen could be seen only under the oil immersion lens. The formation of sperm cells in *Vallisneria* (Witmer, 1937) and *Elodea* (Wylie, 1904) is similar to that in *Blyxa*, but there the sperm cells are spindle-shaped. A spindle-shaped generative cell has been figured for *Ottelia* (Narasimhamurthy, 1935).

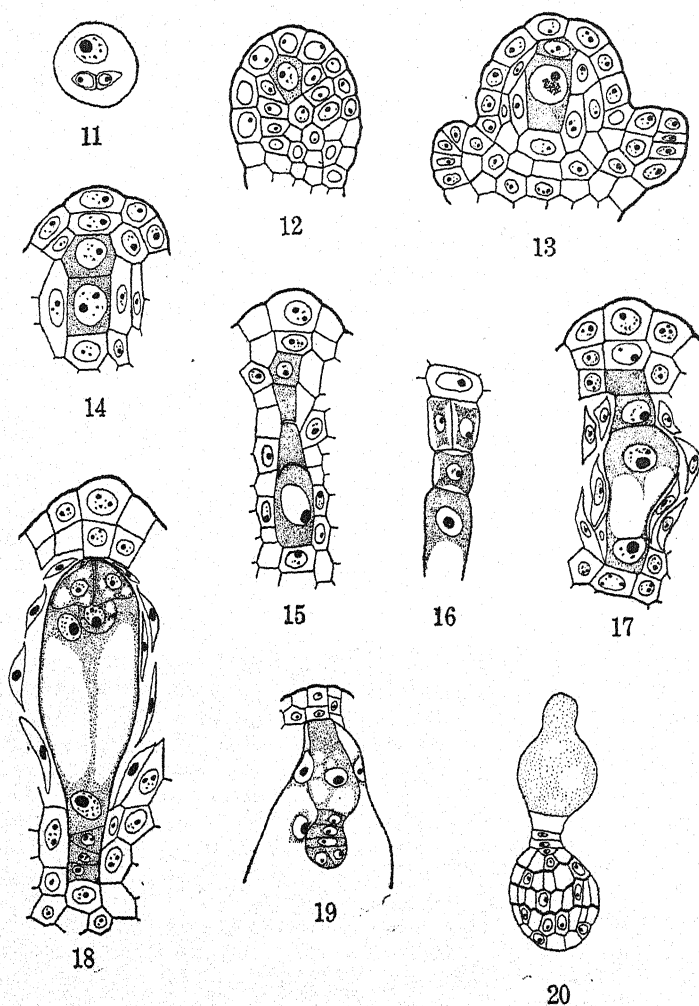
THE OVULE AND MEGASPOROGENESIS

The ovules arise regularly in a line in the furrows of the ovary (Fig. 4). The longitudinal sections of the ovary show wavy ridges in a line marking out the primordia of the ovules. Though *Vallisneria* also has a tri-carpellary ovary as *Blyxa*, the origin of ovules there, seems to be very irregular.

The ovule primordia are all directed towards the top of the ovary though they are horizontal to begin with. The hypodermal archesporium becomes conspicuous in these projections (Fig. 12), even before the initials of the integuments appear. Before the second integument begins to grow, the primary archesporial cell has already cut off a narrow parietal cell and begun to enlarge to prepare itself for the meiotic divisions (Fig. 13). Though in the majority of the Helobiales, the formation of a parietal cell is the rule, there are a few exceptions like *Sagittaria lancifolia* and *Echinodorus rostratus* (Schnarf, 1931). In Hydrocharitaceæ the formation of a parietal cell occurs in *Vallisneria* (Rangasamy, 1934; Witmer, 1937), *Enalus* (Kausik, 1940) and *Elodea* (Wylie, 1904). In *Ottelia*, however, (Narasimhamurthy, 1935) the archesporial cell functions directly as the megaspore-mother cell. Only the epidermal cells divide to make the mother cell deep seated. The parietal cell formed in *Blyxa* does not undergo any further division and remains as a single cell even in the later stages of the seed. But the fate of these parietal cells in other plants seems to be different. In *Vallisneria* (Rangasamy, 1934; Witmer, 1937) the parietal cell undergoes one or two divisions, while in *Enalus* (Kausik, 1940) the parietal cell divides first by an anticlinal division and later by periclinal divisions to form a regular parietal tissue at the micropylar end of the ovule.

The megaspore-mother cell divides to form two cells of which the lower is larger than the upper (Fig. 14). The two resulting cells divide once again to form a linear tetrad (Fig. 15), of which the cell at the chalazal end forms the embryo-sac. T-shaped tetrads of megaspores are also seen (Fig. 16). Cases of irregular division with the formation of only two disorganising megaspores, instead of the usual three have also been noted (Fig. 17). In this connection it is interesting to note the formation of tetrahedral type of megaspore tetrads both in *Vallisneria* (Witmer, 1937) and *Enalus* (Kausik, 1940).

The lowermost megaspore which forms the embryo-sac enlarges greatly at the expense of the surrounding cells (Fig. 17). The



Text-figs. 11-20. *Blyxa echinosperma*.—Fig. 11. Pollen with tube nucleus and two sperm cells ($\times 800$). Fig. 12. Nucellar projection with hypodermal single archesporial cell ($\times 800$). Fig. 13. Parietal cell formed from archesporium with M.M.C. ($\times 800$). Fig. 14. First Division in M.M.C. with parietal cell above ($\times 800$). Fig. 15. Linear tetrad of megaspores ($\times 800$). Fig. 16. T-shaped tetrad of megaspores ($\times 800$). Fig. 17. Two-nucleate embryo-sac with two megaspores above ($\times 800$). Fig. 18. Fully formed embryo-sac with a single parietal cell above ($\times 800$). Figs. 19-20. Developmental stages of Embryo ($\times 400$).

disorganisation of cells does not take place uniformly all round the embryo-sac. The chalazal end of the sac shows the surrounding cells perfectly in tact. The result of this type of development is the formation of a flask-shaped embryo-sac characteristic of

Helobiales with its neck directed towards the chalaza. The embryo-sac is of the normal eight-nucleate type. The egg apparatus consists of two synergids pointed at the apex and a longer egg. All the three are vacuolate. The antipodal cells are well formed and organised in the chalazal pouch of the embryo-sac. The antipodals persist for some time during the enlargement of the embryo, but they are not very conspicuous as to take part in any feeding function. The polar nuclei remain at their respective poles for a very long time and migrate towards each other only just before the entry of the pollen tube (Fig. 18).

THE EMBRYO

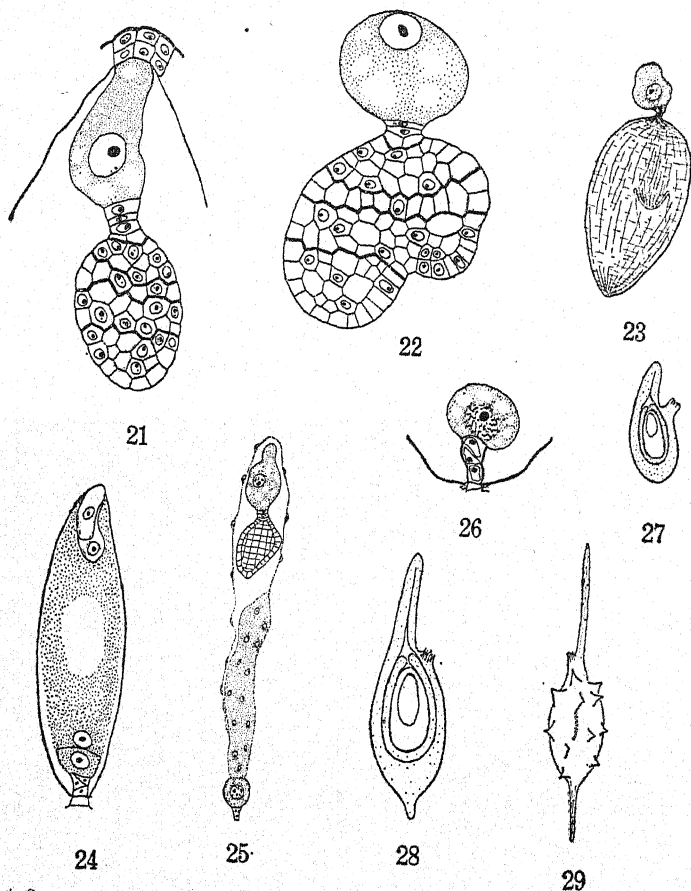
The oospore before division elongates in the direction of the chalaza. The first division in the oospore results in the formation of an elongated basal suspensor cell and an embryonal cell. This basal cell begins to enlarge in size by accumulating nutrient matter and persists till a very late stage (Fig. 19). It is interesting to note that in some of the intermediate stages of development of the embryo, the basal cell will be as large as the embryo itself. This basal cell is characteristic of the group Helobiales, being prominent in all genera. A pro-embryo of four cells like *Vallisneria* (Rangasamy, 1934; Witmer, 1937), is formed (Fig. 19), before a vertical division in the terminal cell occurs. Intercalary divisions in the suspensor cells of the pro-embryo are very common. These keep on increasing the number of tiers in the pro-embryo. Figs. 20 and 21, show a slightly advanced stage of the embryo, whose cells are formed from four tiers. The lower four cells, excluding the large basal cell, form the suspensor. This kind of apportioning of cells of the different tiers to the formation of the embryo is evident by (Fig. 22), which shows the massive tissue of the first and topmost tier going to form the cotyledon, the second tier to the plumule, tiers three and four to the hypocotyl, and the fifth to the radicle. This very well corresponds with the organisation in the embryo of *Sagittaria* (Schaffner, 1897), which can be taken as the typical embryo for the Helobiales.

Whole mounts of the later embryos could easily be made and Fig. 23, represents one of these. This was stained with eosin and mounted under balsam. Here the basal cell is still prominent. The three suspensor cells also are discernible showing how persistent they are. The suspensor does not get easily broken as in *Vallisneria* (Witmer, 1937; Rangasamy, 1934) and *Sagittaria*, (Schaffner, 1897). All the cells of the embryo get filled up with starch grains, making the embryo very hard and brittle. The base of the cotyledon forms a sheath around the plumular tip leaving an opening only on one side.

THE ENDOSPERM

The primary endosperm nucleus which is situated low in the embryo-sac divides earlier than the oospore at the micropylar end.

The two daughter nuclei are separated by a thin wall, which also divides the embryo-sac into two chambers, the larger upper and the smaller lower chamber (Fig. 24). The lower chamber persists as such without further increase in size, whereas the nucleus of the upper chamber after migrating further up, divides by several free nuclear divisions. This course of endosperm formation is common to all Helobiales. The variation appears to be only in the time that elapses between the division in oospore and the division in the



Text-figs. 21-29. *Blyxa echinosperma*.—Figs. 21-22. Embryonal stages ($\times 400$). Fig. 23. Whole mount of teased embryo from seed. ($\times 80$). Fig. 24. Division in primary endosperm nucleus ($\times 400$). Fig. 25. Later embryo-sac with chalazal chamber of endosperm attached to antipodal pouch, endospermic plasma membrane with nuclei and embryo above ($\times 140$). Fig. 26. Chalazal endosperm cell pressed against antipodal pouch ($\times 500$). Fig. 27. Sketch of ovule with integuments covering. ($\times 40$). Fig. 28. Sketch of a seed with micropylar and chalazal projections of outer integument ($\times 24$). Fig. 29. Seed with both projections, funicle and echinate spines ($\times 13$).

primary endosperm nucleus. In *Vallisneria* (Witmer, 1937) the divisions are simultaneous, whereas in *Enalus* (Kausik, 1940) the primary endosperm nucleus divides earlier than the oospore. In *Elodea* (Wyllie, 1904) the division of the endosperm nucleus is delayed until a two-celled embryo is formed.

The endosperm nuclei in the upper chamber organise themselves to form with cytoplasm a peripheral plasmatic layer without cell walls. This membranous layer of cytoplasm with the free nuclei gradually gets used up and disappears with the developing embryo. The lower endosperm nucleus does not take part in nourishing the embryo to any extent. The cell membrane formed during the first division of the endosperm nucleus encircles the lower nucleus to form a cell which (Fig. 26) presses against the lower pouch of the embryo-sac containing the three antipodal cells. Such structures persist only for some time during the development of the embryo. A similar antipodal pouch but without the endosperm cell has been drawn for *Ottelia* (Narasimhamurthy, 1935).

THE SEED

The nourishment for the embryo in the embryo-sac is from (1) the endosperm (plasma membrane with nuclei), (2) the enlarged basal cell, (3) the enriched cells of the surrounding nucellus. No kind of haustorium is observed either at the chalazal end or the micropylar end. The embryo occupies all the space of the seed with the disorganisation of the entire nucellus. The outer coat of the seed develops spine-like projections (Fig. 29), giving an echinate appearance to the seed. The testa just covers the micropylar end of the ovule at the time of fertilisation (Fig. 27), but later it overgrows the micropyle to form a very long tail-like projection which is the longer of the two tails found on the seed. From the chalazal end also a similar shorter extension (Fig. 28), of the testa is found.

CONCLUSIONS

The microsporangium of *Blyxa* shares in common with those of *Elodea* (Wyllie, 1905) and *Vallisneria* (Rangasamy, 1934; Witmer, 1937) the possession of a single wall layer, coming between the tapetum and the epidermis. *Ottelia* (Narasimhamurthy, 1935) however has two wall layers instead. All of them possess a well organised tapetum. The origin of the tapetum in *Blyxa* as in *Ottelia*, *Vallisneria*, and *Elodea* is from the parietal layer. The tapetal plasmodium is common for all these genera.

The parietal cell formed in the ovule from the archesporium does not undergo any division either periclinal or anticlinal but remains as such in the mature ovule. In this respect it differs from those of *Vallisneria*, *Enalus*, and *Elodea*, who all form a parietal tissue at the micropylar end of the ovule.

The chalazal endosperm cell pressed against the pouch of the embryo-sac containing the antipodal cells is rather a peculiar structure in *Blyxa echinosperma*. This has been noticed in many embryo-sacs and thus could be stated to be of definite occurrence in the plant

The function of a suction organ is attributed to this kind of antipodal end in *Enalus* (Kausik, 1940) and *Ottelia* (Narasimhamurthy, 1935), but this cannot be said to be true of *Blyxa*, because the cells of the nucellus below this antipodal end are not particularly rich in contents or in any way modified. Such pouch-like structures lie loose at the base of the embryo-sac without any support of tissue all round, the embryo-sac having enlarged at the base also later. The lower endosperm cell does not enlarge to indicate that it takes part in a feeding function.

The embryo formation in the whole of Hydrocharitaceae is of *Sagittaria*-type, all of them agreeing in the general principle of development. All the genera have the large basal cell, four to five tiers of proembryonal cells going to form the parts of the embryo; the second tier particularly forming the plumule. Intercalary divisions are common in all the cells of the proembryo except the large basal cell. It is interesting to note that the embryo of *Enalus* (Kausik, 1940) alone does not have a suspensor of even a single cell, in the earlier stages at least, though a suspensor of three cells is a common feature in the other genera *Ottelia* (Narasimhamurthy, 1935), *Elodea* (Wylie, 1904), *Vallisneria* (Witmer, 1937; Rangasamy, 1934), and *Blyxa*. In *Enalus* the enlarged basal cell abuts directly against the embryo feeding it much better than through a suspensor.

SUMMARY

(1) The mucilaginous ducts are established earlier than the vascular system in the flower.

(2) The male archesporium cuts off a parietal layer which by periclinal division forms the tapetum. The tapetal cells are uni-nucleate and later form a periplasmodium. The pollen has two male cells in addition to the tube nucleus.

(3) The female archesporium is single and hypodermal. It cuts off a parietal cell which does not divide any further. The tetrads are linear or T-shaped. The chalazal megaspore forms the embryo-sac which has the normal eight-nucleate type of development. The fusion of the polars is very much delayed; even the migration towards each other, taking place after the entry of the pollen tube.

(4) The primary endosperm nucleus divides to form a chalazal and a micropylar chamber of the embryo-sac. The lower chalazal chamber does not help in the nourishment of the embryo.

(5) The upper chamber of the endosperm without forming cells develops a plasma membrane with free nuclei. This is fully used up by the time the embryo is half developed.

(6) The embryo has a large basal cell and a suspensor of generally three cells in addition to its other parts. The method of development is of *Sagittaria*-type. The embryonal cells are full of starch grains.

(7) The ovules are anatropous and pointed upwards in the ovary. The two integuments cover up the ovule prior to the formation of the egg-apparatus and later the outer integument by its abnormal growth forms a beak-like projection at the micropyle. The seed is echinate and has another long integumentary growth at the chalaza.

In conclusion, I have great pleasure in expressing my grateful thanks to Dr. M. A. Sampathkumaran, who provided all facilities and helped me during the course of this investigation.

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HIGHER FUNGI OF THE PANJAB PLAINS

III. The Gasteromycetes

BY SULTAN AHMAD, M.Sc.

Biological Laboratory, Govt. College, Rohtak

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IN this third paper of the series dealing with the higher fungi of the Panjab plains, some species which are new to science, and others new to this region are described. The occurrence of species like *Queletia* sp., *Lycoperdon echinella*, *Podaxon calyptratus*, *Phellorinia strobilina* and *Dictyophora irpicina* is remarkable suggesting what a variety is found when even a small region is intensively explored.

The Genera *Queletia*, *Schizostoma* and *Tylostoma*

The genera *Queletia*, *Schizostoma* and *Tylostoma* form a fairly natural group within the family Tylostomaceæ which has already been defined by the writer (1939).

The genus *Queletia* was proposed by Fries in 1871 with a single known species *Queletia mirabilis* Fries. Very little is known about it and it is regarded as "a mystery of the puff-ball world" and as "a rare and interesting plant of curious distribution and habits". It is characterised by its large size, scaly stipe and the irregular breaking up of the peridium.

The genus *Schizostoma* was proposed by Ehrenberg in MS. on a plant collected from Equatorial Africa, and named by him *Schizostoma laceratum* Ehrenb. This species has since been collected from California and the Panjab. As the name was pre-occupied by a genus of the family Cyttariaceæ of the Ascomycetes, it was transferred by Fries (1829) to *Tylostoma*, as *Tylostoma laceratum*. Lloyd (1904) on the other hand stated that the plant deserved a generic rank, separate from *Tylostoma*.

The species (*Tylostoma laceratum*) with its entirely different mode of dehiscence, occupies an anomalous position in the genus *Tylostoma*, all the species of which genus possess a definite mouth. On the other hand, it agrees with the genus *Queletia* in the manner of dehiscence and gleba characters, and differs only in the smaller size and the striate non-scaly stipe.

A species of the genus *Queletia* recently found as of common occurrence in the Panjab plains throws additional light on the affinities of this genus with the genus *Schizostoma sensu* Ehrenberg. The Panjab species shows all gradations from smaller size and striate stipe of *Schizostoma* to the large size and scaly stipe of typical *Queletia*. In fact the resemblance in some cases is so close that at

times it becomes difficult to distinguish between the two, excepting by gleba colour.

From a comparative study of some hundreds of plants found in the Panjab plains the writer finds that the affinities of the two are so close to each other that they should no longer be kept separate. With the new species proposed below as the connecting link, it becomes apparent that the genus *Schizostoma* is co-generic with *Queletia* and the species is renamed as follows :—

Queletia laceratum (Ehrenberg) Comb. nov.

Syn. *Schizostoma laceratum* Ehrenberg; *Tylostoma laceratum* (Ehrenb.) Fries

A New Species of *Queletia*

In August 1939 a plant resembling *Queletia mirabilis* in size and mode of dehiscence was collected by the writer in sand-hills near Jhang. It presented several important points of difference from that species, which is only known from France, United States and England, growing on soil or tan bark.

According to White (1901) the stipe in *Queletia mirabilis* is solid and reddish brown, both within and without. The spores are pedicelled and coarsely warted, 5–6 μ in diameter. But Coker and Couch (1928) report that they found spores measuring upto 6–9 μ in diameter in Dr. Herbst's specimens from Pennsylvania. The capillitium is stated to be hyaline according to Lloyd (1903) and the original description of Fries (quoted by Coker and Couch, 1928), but White (1901) records it as pale yellow.

In the Panjab plant the stipe, however, is hollow, stuffed and always white. The spores are perfectly smooth, without a pedicel and 4–6.5 μ in diameter. The capillitial threads are definitely coloured, only rarely pale yellow. At the same time there is nothing curious about the distribution of this plant as it is fairly common in the plains and frequents sandy soil. There seemed to be enough justification therefore to consider it as a new species and the name *Queletia Mundkuri* is proposed for it.

30. *Queletia Mundkuri* S. Ahmad Sp. nov.

Peridium globosum, aliquando irregulare, apice latius usque ad 5 cm. latum et 3 cm. altum. Exoperidium ex arenaceo tegumento, quod dum planta surgit e terra, complete abjicitur, endoperidio perfecto glabro relicto. Endoperidium sive sordido-album sive rubro-brunneum, potius fragile, in magnas irregulaesque valvas prorumpens dum dehiscit. Stipes usque ad 15 cm. longus et 2 cm. latus, perfecte albus, rudibus squamis obductus; cavatus infarsus, sine volva, etsi in quibusdam exemplis, sed raro, habeatur structura aliqua vovæ similis; singulari rhizomorpha humo defixa, vel pluribus rhizomorphis, quibus humi particulae in densam molem constringuntur. Stipes habitualiter squamosus, aliquando tamen

sine squamis ; squamæ tenues, fibrillosæ vel potius rigidæ, crispatæ et fragiles.

Gleba nigro-rubida ; sporæ leves, globosæ vel sub-globosæ, diametro 4-6.5 μ . Capillitium ex filamentis longis, ramosis, aseptatis, colore pallido-flavido, in brevibus fragmenta extremitatibus clausis dirumpens.

Habitat.—Solitarie arenoso solo.

Locus.—Sangla Hill ; Jhang ; Rohtak ; Leg. Sultan Ahmad.

Peridium globose, sometimes irregular, broader at the apex upto 5 cm. in diam. and 3 cm. in height. Exoperidium a sandy coat which falls away completely as the plant emerges out of the ground, leaving the endoperidium perfectly smooth. Endoperidium dirty white or reddish brown, dehiscing by irregularly breaking up into large valves, rather fragile.

Stipe upto 15 cm. long and 2 cm. thick, perfectly white, coarsely scaly ; hollow, stuffed, without any volva, very rarely a structure resembling it present in some specimens ; attached to the ground by a single thick rhizomorph or a number of them binding the soil together in a thick mass. The stipe usually scaly but non-scaly also occur ; scales thin, fibrillose or rather stiff, curled and fragile.

Gleba dark brown ; spores globose or sub-globose, 4-6.5 μ in diameter, smooth ; capillitium consisting of long branched, aseptate threads, breaking into short fragments with closed ends, coloured or rarely pale yellow.

Habitat.—Solitary in sandy soil.

Locality.—Sangla Hill ; Jhang ; Rohtak (Figs. 1 and 2).

Type.—Deposited in writer's Herbarium and Herb. Crypt. Ind. Orient. of the Imperial Agricultural Research Institute, New Delhi.

Calvatia Fries

31. *Calvatia lilacina* (Berk. et Mont.) Lloyd, *Myc. Notes*, I ;
Lyc. Aust., 1905, 35 ; Syn. *C. cyathiformis* (Bosch.) Morgan

Plants sub-globose to pyriform, 5.5 cm. in diam. and 6 cm. in height, attached by a short root. Exoperidium smooth or slightly scaly, cream to bay-brown, thin fragile fugacious ; endoperidium brown, thin at maturity flaking away irregularly to expose the spore mass. Sterile base well developed, persistent, cellular, separating from the gleba by a definite diaphragm. Gleba at first compact and white becoming deep purple brown and pulverulent at maturity. Spores globose 5-7 μ in diameter (including the spines) verrucose ; capillitium of long branched septate threads, rounded and narrowed at the joints. There are no pits seen on the walls of capillitium threads even under oil immersion as are described by Coker and Couch (1928).

Habitat.—Solitary on sandy soil. Common.

Locality.—Gurdaspur.

A very widely distributed species, characterised by deep purple brown colour of the gleba, presence of diaphragm and verrucose spores.

Lycoperdon (Tourn.) Pers.

32. *Lycoperdon echinella* (Pat.) Comb. Nov.

Syn. *Bovista echinella* Pat., *Bull. Soc. Myc. Fr.*, 1891, 7, 165;

Bovistella echinella (Pat.) Lloyd, *Myc. Writ.*, 1906, 2, 262

Peridium globose or sub-globose, 0.5–1 cm. in diam. and upto 0.7 cm. in height, attached to the ground by a very short mycelial base. Exoperidium furfuraceous, peeling off partially and leaving the endoperidium smooth. Endoperidium yellowish or bay-brown, opening by a small torn apical aperture. Sterile base absent.

Gleba olivaceous; capillitium of long sparsely branched, very rarely septate threads $2.25\text{--}4.5\ \mu$ in diameter; spores globose $4.5\text{--}5.2\ \mu$ in diam.; pedicellate, pedicel acuminate upto $6.5\ \mu$ in length, very slightly tinted; epispore yellowish brown, smooth.

Habitat.—Solitary on the ground among patches of *Funaria hygrometrica* Rare.

Locality.—Ladhar, Sheikhpura.

A very small and rare species of *Lycoperdon* characterised by small size, smooth pedicellate spores and absence of sterile base.

It was originally described as *Bovista echinella* by Patouillard (1891) but was later transferred to the genus *Bovistella* by Lloyd (1906) on account of its having a well-developed rooting base, the only character which separates the genus *Bovistella* from *Bovista*.

As defined by Lloyd (1906 b) the genus *Bovistella* includes all the puff-balls with pedicellate spores and a well-developed rooting base. But as understood at present it includes only those species which have the capillitium of short separate threads (Sections I and III of Lloyd), while those having capillitium of long intertwined threads (Sections II and IV of Lloyd) are now put in the genus *Lycoperdon*.

The present species has the typical capillitium of the *Lycoperdon* type and is accordingly referred to that genus. It is a widely distributed species and has been recorded from Ecuador, Jamaica, Mexico, Michigan, Washington and Europe. It is very close to *Lycoperdon trachyspora* (Lloyd) described from India, from which it only differs in having smooth spores (markedly tuberculate in that species).

Podaxon (Desvaux) Fries*

A curious plant growing on the mud roof of a house was collected in the Panjab plains in August 1934. It was growing within the mud and its presence could be made out due to the swelling and cracking of the ground above it. Specimens were sent to Dr. Coker of the North Carolina University, who remarked "this is a very interesting plant and does not fit anything we can find, particularly as you say it is subterranean throughout its life. It seems certainly near *Podaxon*, but all species of that genus are stalked and above ground when ripe".

Since then two more plants have come to light from the same locality, one of which is young and the other fully ripe. These resembled the original plant perfectly in the subterranean habit and in the total absence of stipe. The plant differs only in its habit from the genus *Podaxon* and may be an anomalous form of some other species. The peculiarity may be associated with the peculiar habitat of the plant.

33. *Podaxon subterraneum*? S. Ahmad, Sp. nov.

Peridio oblongo-depressum 6×9 cm., squamulis albidus; irregularita dehiscente; stipes brevis nodosus. Gleba prope niger; sporis globosis vel latus ellipticus, $7.2-12$ vel $10.5-13.5 \mu$, rubicundus vel fuscus, levibus; capillitio paucis, pallido flavido.

Habit.—Subterraneus.

Locus.—Ladhar, Sheikhpura.

Peridium compressed, oblong, 6×9 cm. Exoperidium of small white scales; endoperidium in the ripe specimen dark brown, tough, covered with debris, opening by an irregular rupture. Stipe practically absent, merely a knob-like outgrowth at the base, traversing the gleba as a percurrent columella.

Gleba almost black; spores globose or broadly elliptic, $7.2-12$ or $10.5-13.5 \mu$, epispore thick, reddish brown or dark coloured, smooth, with an apical germ pore; capillitium of few pale yellow threads.

* Some doubt has been expressed about the adoption of the generic name *Podaxon* instead of *Podaxis*. The name *Podaxis* as proposed by Desvaux is of an earlier date, and Massee restored it in 1890, not caring for the grammatical inaccuracy pointed out by Fries who on this account had previously changed it to *Podaxon*. Massee (1890) says that a "generic name is only of symbolic value, it is best to adhere to the original symbol, whereby avoiding the inevitable complication following any tampering with the original name". More recently Fischer (1933) has also adopted the name *Podaxis*, but the writer prefers to follow Cunningham (1932) who cites both Desvaux and Fries as authorities, retaining at the same time the grammatically correct name *Podaxon*. Due to oversight the writer in the earlier paper failed to cite Desvaux's name along with that of Fries.

Habit.—Subterranean.

Locality.—Ladhar, Sheikhpura.

Type.—Deposited in Herb. Crypt. Ind. Orient. of the Imperial Agricultural Research Institute, New Delhi.

34. *Podaxon calyptratus* Fries, *Syst. Myc.*, 1832, 3, 63.

Syn. *P. loandensis* Welwitsch and Currey (1868);

P. axata (Bosch.) Massee (1890); *P. Muelleri* P. Henn.;

P. Gollani P. Henn. §

Closely resembles *Podaxon pistillaris*, a very common plant in the Panjab plains, in form and habit. The olivaceous or black colour of the gleba of this species presents a marked contrast with the reddish brown colour of the other one. Gleba very fragile, consisting of a mass of spores with a very scanty capillitium. Capillitium hyaline, sparsely septate and rarely branched threads. Spores globose or obovate, upto $9 \cdot 0$ – $15 \cdot 5 \mu$; epispore thick, chestnut brown, smooth, perforate apically and with or without a basal hyaline pedicel.

Habitat.—Solitary in sandy soil. Common.

Locality.—Rohtak.

The species is characterised by the olivaceous or dark coloured fragile gleba, and practical absence of capillitium which consists of hyaline threads.

35. *Phellorinia strobilina* (Kalchbr.)

Kalchbr. & Oke. Grev., 1880, 9, 4

Plants may vary from 4·5–15 cm. in total height; the peridium from 2·5–6 cm. in height and 0·8–4 cm. in diam.; while the stipe may range from 2–8 cm. in length and 0·8–4 cm. in diam. Exoperidium in the form of large thick pyramidal warts, the only feature which separates it from *P. inquinans*.

Gleba with persistent fascicles of basidia; pale yellow to brown; spores globose, a few ellipsoidal, sometimes with a short hyaline pedicel, pale yellow under the oil immersion, 5 – $7 \cdot 5 \mu$ in diam. covered with flat topped coarse warts which give a reticulate appearance.

Habitat.—Solitary or in groups in sandy soil. Sometimes two plants are seen growing from the same rhizomorph. Common.

Locality.—Rohtak.

§ *Podaxon Gollani* was described by P. Hennings (1901) from specimens collected by Wm. Gollan at the Saharanpur Botanical Garden, India. The specimens were mostly unripe, and as pointed out by the author were closely allied to *P. carcinomalis*.

According to Cunningham (1932) the description agrees closely with that of *P. calyptratus*, with which it is probably synonymous.

The plant was extensively collected in all stages of development in Rohtak in July 1939 by the writer's students. As the plants were not carefully gathered, none of them showed the characteristic scales which readily separate it from the closely allied species, *Phellorinia inquinans* and was erroneously referred to that species by the writer (1939).

The specimens collected by the writer in March 1940 from the same locality leave no doubt that they belong to *Phellorinia strobilina*, which as Cunningham points out differs from *P. inquinans* 'in the much larger size of the plants and the nature of the exoperidium, which is covered with large, thick, pyramidal, persistent, zoned scales'. The Panjab plant is found to be very variable as to size, and the scales which are not persistent but sooner or later fall off, exposing the smooth white endoperidium.

36. *Dictyophora irpicina* Patouill., *Bull. Soc. Myc. France*,
1898, 14, 190; *Dictyophora merulina* Berk.;
Clautriavia merulina (Berk.) Lloyd

A common species in Java, Sumatra and Ceylon, but so far the Indian record is based on a stray specimen collected by Rev. E. J. Blatter from Panchgani, Bombay and reported by Narsimhan (1932).

It was erroneously referred to *Itajahia galericulata* in an earlier paper by the writer (1940). The pileus surface is strongly folded and convolute and is not in the form of plates as in *Itajahia*; this being the only difference in the two plants.

The plant has so far been collected from Rohtak and Sangla Hill. The Rohtak specimens are very large and in abnormal specimens two plants are seen emerging out of a common volva. Sections reveal a well-developed primordial tissue between the stem and the pileus. In specimens from Sangla Hill on the other hand this primordial tissue is totally absent.

The plants from the two localities also differ as to habitat. The Rohtak plants are buried in sand while young, but the Sangla specimens grow on rich humus soil under *Salvadora* trees.

A veil is found in several specimens as a thin transparent membrane hanging from under the pileus and also attached to the stem here and there, but the indusium is never found in any plant.

Tylostoma volvulatum Borsch. is a very common plant of wide distribution, found in North Africa, Central Asia and Central Europe. The writer (1939) has reported it from the Panjab plains, but it has since been found to be of very common occurrence especially in sandy wastes round about Rohtak. At the same time the writer has found it to be a very variable species. The writer thinks it advisable to supplement the description already presented.

"Peridium globose or depressed globose, 1.2–5.2 cm. in diam. and 0.7–2.6 cm. in height; exoperidium a sandy case 1 mm. in thickness, composed of sand particles and hyphæ, falling at maturity leaving some sand particles attached to the endoperidium, which also fall in course of time, leaving the endoperidium smooth. If young specimens are unearthed, the exoperidium exhibits a fluffy white mycelial outgrowth; the threads forming it grow over the sand particles and entangle them to form the sandy coat. Endoperidium pure white or light chocolate in colour, opening by a definite circular mouth bounded by a slightly raised margin, or by a slightly raised irregular rupture which in nature becomes an elongated or perfectly circular opening. Generally there is a solitary mouth, but sometimes as many as 5 may be seen.

Stipe 0–15 cm. in length and 0.4–1.8 cm. in diam., curved, straight or tortuose; smooth, striate or broken into few very large caducous scales, stuffed, white in colour uniform or tapering to the base, with a very large volva at the base, upto 2.5 cm. in diam., attached to the ground by a branching root cord from 0.6–1.2 cm. in thickness and several centimetres in length. Gleba characters as already described; characterised by the coloured capillitium and smooth spores."

In the light of variations recorded above, one can confirm Lloyd's statement (1906 a) that "*Tylostoma volvulatum*, *T. americanum* and *T. caespitosum* are very close to each other and may be safely regarded as the forms of the same species". The Panjab plant is typical *T. volvulatum*, as it has the same coloured capillitium, solitary irregular or elongated mouth and smooth spores. It approaches *T. americanum* in size, smooth spores and occasionally possessing several mouths. It differs from *T. caespitosum* only in the coloured stipe and rough spores characters which vary even in the same species.

ACKNOWLEDGMENT

The writer wishes to express his gratitude to Dr. B. B. Mundkur for his invaluable help throughout this work.

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EXPLANATION OF PLATE II

Fig. 1. *Queletia Mundkuri*—Specimen from Jhang. (Nat. size)

Fig. 2. *Queletia Mundkuri*—Specimen from Rohtak. (Nat. size)





FIG. 1

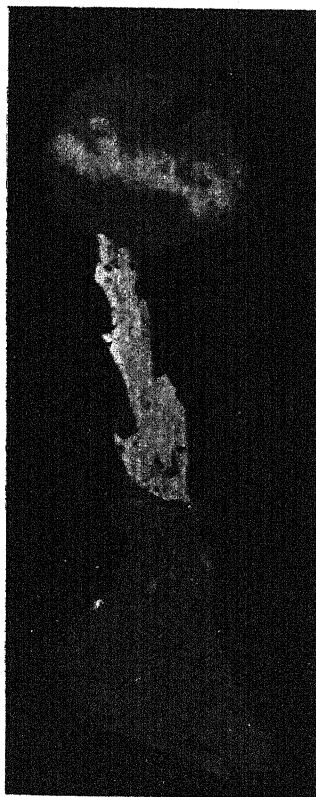
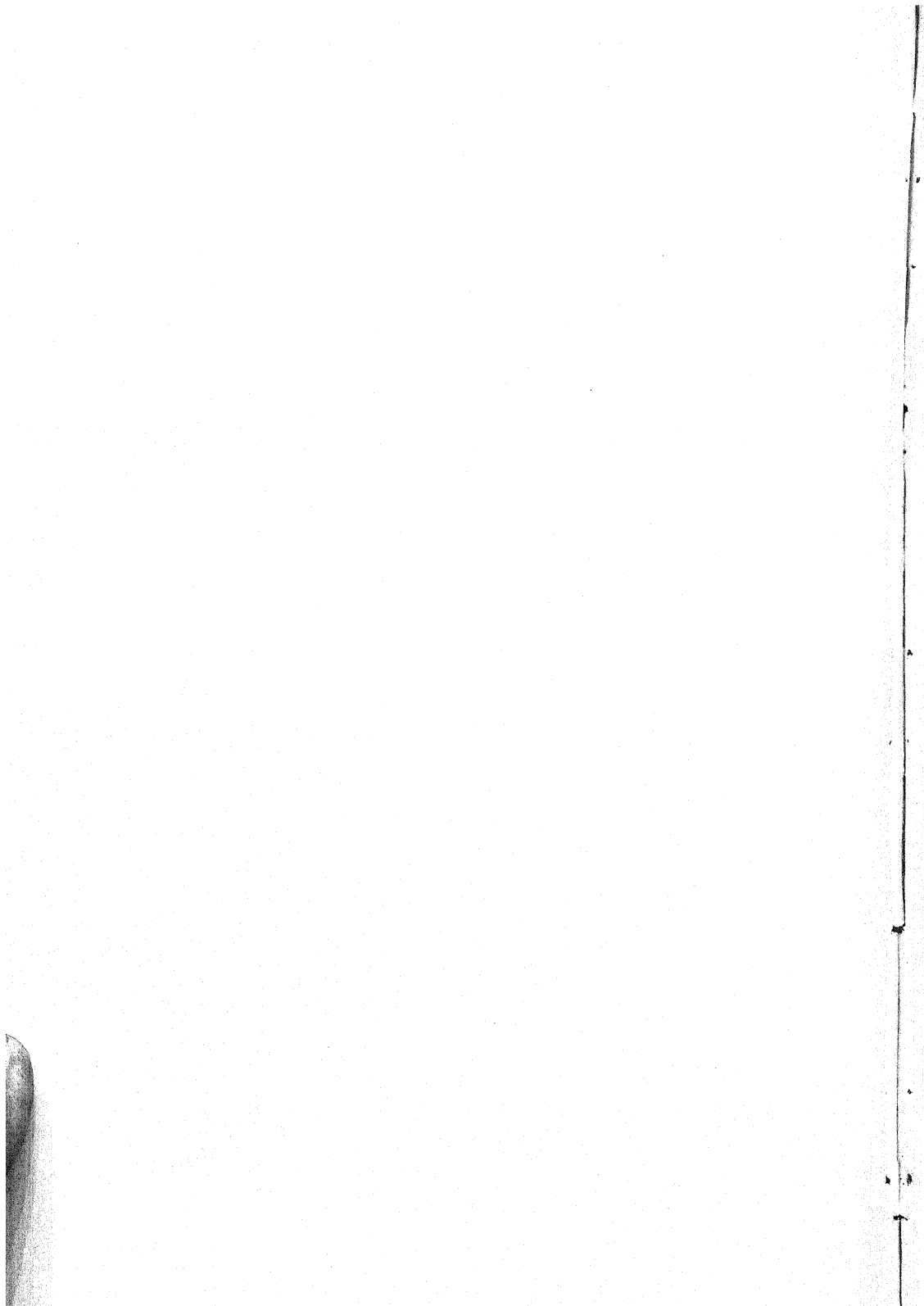


FIG. 2

SULTAN AHMAD—HIGHER FUNGI OF THE PANJAB PLAINS



A PLEA FOR BETTER CO-ORDINATION OF BOTANICAL WORK IN INDIA*

BY H. CHAUDHURI, D.Sc., LONDON, Ph.D., D.I.C.

*Head of the Department of University Teaching in Botany,
and Director, Kashyap Research Laboratory,
Panjab University, Lahore*

IN his Presidential Address at the Madras meeting last year, Rai Bahadur Professor K. C. Mehta suggested that we should take up without delay "some if not all the line of work that remains untouched so far". Among other things, he suggested the appointment of nine Standing Subject Committees and three Regional Committees. I considered many of these suggestions important and practical and I have tried to give effect to some of these. You know, I have sent resolutions for incorporating new rules which you will be asked to consider to-day. I have proposed the appointment of the following sub-committees for the present, *viz.* :—

- (a) Phytosociology and Plant Physiology Sub-committee,
- (b) Plant Genetics Sub-committee,
- (c) Phytopathology Sub-committee, and
- (d) Economic and Industrial Sub-committee.

Each sub-committee is to have its own Secretary and 2-3 members with power to co-opt more whenever necessary. These sub-committees will periodically bring to the notice of the members, through the pages of our Journal, the published work, as well as reports of work in progress by workers in this country. They shall also publish very brief reviews of important literature published abroad, specially on newer lines of research and technique and also establish contact with foreign societies. For instance the Sub-committee on Phytosociology and Plant Physiology will establish contact with the British Ecological Society, American Ecological Society, International Association of Phytosociology, American Society of Plant Physiologists and such other societies in foreign land and supply information to the members on mapping of vegetation and other ecological and physiological matters. The Economic and Industrial Sub-committee, besides other functions, will bring to the notice of the proper authorities, may be Government or State or Private persons or Companies, researches of commercial and industrial importance. The members of this Sub-committee will take note of researches of potential monetary value, and research workers also will bring to their notice any result likely to be useful. When this Sub-committee functions properly, Industrialists will no

* Presidential Address, Indian Botanical Society, Twentieth Annual Meeting, Benares, January 3, 1941.

doubt approach them for the solution of any problem requiring the knowledge of any special branch of Botany. Instead of Regional Branches, I have proposed amendment of our rules so as to provide for local branches at different centres of learning and for affiliating societies having similar aims and objects to those of our Society. Holding of meetings of members from different centres of a regional branch even once or twice during the year will not be a feasible one, but if local branches were started with six or more members of the parent society and if these branches were given the right to enroll local student members at a nominal fee to meet the cost of expenses of the local branch, more meetings could be held which would no doubt interest more people in the work of the Society. Activities of these branches will be published in the Journal of our Society.

By affiliating other societies having similar aims and objects to those of ours, we shall be helping each other. We shall bring to the notice of the botanists in different parts of India and abroad through the pages of our Journal the work done by them. We shall have no control over the management of the affiliated societies. I have suggested that they shall pay Rs. 25 as affiliation fee and receive 2 copies of our Journal free, or if you so decide, they shall pay only a nominal fee of Rs. 5 p.a. for affiliation. All activities of these societies will be published in the pages of our Journal.

I have no doubt you will give the best consideration to the above proposals.

For the advancement of Botany not only work on co-operative lines has to be taken up in every field of botanical studies and research but effort has to be made to unite the various Botanical interests. For this purpose, and also to prevent wastage of time and energy, our work has to be properly planned and this could only be done by small sub-committees. In this Address, I intend to lay great emphasis on co-operation, collaboration and co-ordination in scientific work.

Co-operation in the field of scientific work is very important. I have as a researcher felt this lack of co-operation very much. I am not complaining of lack of co-operation between individual workers in a place or between workers in one University and another but between workers in the Government departments and workers outside it, to the great detriment of progress of scientific research in this country. I shall make this point clear by referring to some concrete cases of which I have got personal experience. Many of you are no doubt aware of the existence of a Board of Agriculture and Animal Husbandry, which has a "Crops and Soils Wing". Meetings of this Wing are arranged in alternate years by the Imperial Council of Agricultural Research and scientific papers are read and discussed; but these meetings are practically confined to the workers in the Government Agricultural Department. Once in a letter to the Agricultural Commissioner, I wrote "I expected the I.C.A.R. to invite workers in the University to take part in these meetings. After all, the research of Agricultural importance is not confined

within the precincts of the Agricultural departments either in this country or abroad. Work of far-reaching agricultural importance has been done by the workers in the Universities and you will agree that even in this country it is desirable to bring our work to the notice of the agriculturist and other agricultural research workers. The I.C.A.R. should give us the same facilities as are given to the workers in the agricultural departments in this respect." "So far as University workers are concerned," the Agricultural Commissioner in his reply informed me, "the procedure followed has been to invite to the Crops and Soils Wing,

(i) those workers who are nominated by the Inter-University Board in accordance with the constitution of the Wing as laid down by the Government, and

(ii) particular individuals invited to take the lead in a debate on a particular subject in which they happen to be specialists." He further added, "When the Crops and Soils Wing meeting is held away from Delhi and in a University town, then workers of the local University are often invited to join the Wing as you were at the Lahore meeting." Perhaps in many of the aspects discussed at the Crops and Soils meetings, more work is being done in the different Universities in India, and these workers would very much like to bring to the notice of the Crops and Soils Wing their work and also take part in the deliberations; but this is being prevented on account of the narrow outlook of the authorities who cannot realize that they are thereby hampering the progress of the science and the real interest of our country. Not only was my very legitimate request to be present and communicate papers turned down, but even the request for a copy of the draft Proceedings of the Wing with abstracts of the papers read and discussed was refused. The Agricultural Commissioner wrote "As you are not a member of the Crops and Soils Wing of the Board of Agriculture and Animal Husbandry, 1939, it is not possible to send you a copy of the draft proceedings," but he kindly added "The printed proceedings will of course be available as a priced publication for any one who desires to purchase it". So even this little co-operation and courtesy to a scientific worker doing similar kind of work are denied. It will be for the benefit of the workers both in the Government Agricultural Department and in the University to know each other's work and discuss matters on a common platform. It is needless to emphasise that many aspects of the problems discussed at the Wing meetings are receiving particular attention from the University workers. If these workers are asked to bring their points of view to the notice of the Wing, much benefit would accrue to all concerned. A little departure from the official routine would do no harm.

In the 1937 Crops and Soils Wing meeting at Lahore, which I attended by invitation, I sent a notice that I wanted to move a resolution to the effect that the workers in the Universities should have the same opportunity as other workers in the Government

Agricultural Departments to bring their work to the notice of the Conference. I was informed in reply, "I do not think the resolution you mention is necessary. We welcome all speakers and the University representatives or visitors are very welcome to give us the help of their advice," but actually the position seems to have worsened since then.

In the early days of the I.C.A.R., I undertook an investigation of certain *Citrus* diseases in a scheme financed by them. I think it was one of the very few schemes which were completed within the scheduled time proposed when the scheme was first submitted. When the result of the investigation was published and I returned the equipments purchased with their money, the I.C.A.R. demanded the slides of the sections from which I had made drawings in the publication. This I was loth to do. I maintained that the slides were like the manuscript of my paper, and so must remain with me. I had to get the opinions of scientists from all parts of the world on this point to convince the authorities that those slides should remain with me rather than with anybody else.

Even in the matter of publication of scientific articles my experience of the I.C.A.R. is not a happy one. Several years back I sent a report on samples of soil and water from the paddy fields of Bengal. As the samples had been sent to me by the I.C.A.R., I asked permission to publish the same in some journal as I considered it interesting. The paper, however, was accepted for publication by the I.C.A.R. Several months later, I received a reprint of a paper on the same subject by a chemist who had got quite a different result from mine with an enclosed letter from the I.C.A.R., requesting me that in view of the above paper, I should not press for the publication of my paper. I was in England at that time. When I returned, I searched for my original manuscript, but I could not find the same. Recently, however, I found it, and some of you might have read my note on the Nitrogen fixation in the Rice-field soils of Bengal, in the June issue of '*Nature*' last year.

You are no doubt aware of the important investigations on the epiphytotics of rust on wheat in the plains of India carried out by Prof. Mehta of Agra College. In a symposium on rust problems in India led by Prof. Mehta just three years back at the Jubilee session of the Indian Science Congress at Calcutta, Prof. A. H. R. Buller, F.R.S., of Manitoba, who took part said, "All these recommendations seem to me to be wise and practical and I trust the Government will see its way to carry them out particularly No. 2, which would mean the suppression of wheat crop which is grown on only about 2000 acres." The recommendation No. 2 read, "that in the Nilgiri and Palni Hills the first crop of wheat now sown during April-June should not be sown at all, but should be replaced by some other crop." I said in that discussion, "It seems very important to me that Government should as a test-measure carry out Professor Mehta's suggestion for a couple of years in the

Palni and Nilgiri centres. If successful, the amount of saving will amount to several million rupees." "It should not do to stop with a scientific report." But I am afraid it is going to be a mere report now. Though loss due to rusts amounts to nearly six crores of rupees annually, it is a pity that some more money could not be provided to test this recommendation under Prof. Mehta's direction though eminent scientists thought it sound and practical. I cannot help feeling that if on the Advisory Board, a larger number of University representatives were present, they would certainly recommend the testing of this measure. There is no end of such instances of narrow scientific outlook. Last year one of my research students was working on certain aspects of vernalization of linseed plants. It so happened that there was an epiphytotic of rust on linseed that year and it was noticed in our experimental plots that the intensity of the epiphytotics inversely decreased with the length of pre-sowing cold treatment. The plants which had been exposed for the longest period escaped the disease altogether. I published a note on the same and sent it to the Agricultural Commissioner enquiring if the I.C.A.R. would favour a scheme on the effect of vernalization on disease resistance. I considered the investigation was likely to yield very valuable results. But the reply was "We are already financing one scheme which is dealing with vernalization and I do not think the Advisory Board would be willing to consider another at present." So vernalization was vernalization, it did not matter for them whether it was an investigation on this crop or that crop, whether for the plains or hills, whether stimulating vegetative activity or flowering, whether disease resistance or frost resistance. Their duty was done. One investigation on vernalization had been encouraged! We should see that this kind of attitude is changed.

Regarding schemes submitted by the University workers, I am told, "All schemes receive very careful scrutiny and University schemes are, if anything, *treated more tenderly* than those coming from Governments or States." I wish it were so. But I have found that a University scheme passed by the Provincial Research Council after it had been recommended by the special sub-committee of the Council and forwarded to the I.C.A.R., when it came before their relevant sub-committee, the author of the scheme was invited to come up to Simla to explain his scheme *at his own cost if he liked*. Since the scheme was not to benefit the author personally in anyway, the invitation to attend at his own cost was refused, and there was no one to explain a very technical scheme for the benefit of the members. Government and State-schemes had their representatives, and very often the authors attended at Government expense to explain and advise. I wonder if this was the more tender treatment referred to above.

Many of the schemes, sanctioned and financed by the Government, I think, will not lead to any tangible result and should not have got through but for the present constitution and the procedure followed. I would suggest to the authorities that when schemes

are received by the I.C.A.R. a copy of the same, relating to Botanical matters, may be sent to the Secretary, Indian Botanical Society, who would forward the same to the Secretary of the relevant sub-committee that you are being asked to appoint to-day. The Secretary of the particular sub-committee will circulate the same to its members and forward their recommendation duly. We must also try to get representation on the I.C.A.R. committees for Botany—applied or pure. Happily we have amongst our members, people from all spheres and specialists in different branches. We could easily send representatives by rotation.

Authorities in the Government, who are responsible for selection of subjects for the various competitive examinations conducted by the Federal Public Service Examination, are not favourably disposed towards the biological sciences. Subjects like Botany and Zoology, which were introduced for the first time in the I.A.A.S. and other joint competitive examinations in 1931, were taken out of the list in 1934, because "of difficulty of arranging for such large number of optional subjects," and during the two years these subjects were included in the list, every year one or more of our students successfully competed. Two of them now, I understand, are working as Under-Secretaries in the Government of India. This deletion of the biological sciences from the list has affected our subjects very adversely. The best boys will naturally think of entering for the competitive examinations, and these subjects being ruled out, they do not take up these subjects any more. This callous indifference towards the biological sciences has got to be stopped. We, in the Panjab, have taken up the matter again, and I hope you will also press for the inclusion of these subjects. The importance of these subjects must be recognized. I consider no culture complete without the fundamental knowledge of biology.

I shall now deal briefly with some of the Research institutions relating fully or partly to Botanical Sciences and maintained by the Government of India. Here may be included

- (1) the Imperial Agricultural Research Institute at New Delhi with sub-stations at different places,
- (2) Indian Forest Research Institute and College, Dehra Dun,
- (3) Industrial Section of the Botanical Survey of India, Calcutta,
- (4) The Royal Botanical Gardens, Sibpur, Howrah, and
- (5) The Imperial Sugarcane Station, Coimbatore.

I think the work, in some of these institutions, is going on in a stereotyped fashion. I realize the limitation of research grant and staff in many cases, making it difficult to enlarge the activities of these establishments in the present order of things, but I am of opinion that if the principle of co-operation and collaboration with the Universities were accepted and established and the research work properly co-ordinated, the output of useful work could be

increased considerably. For this I would suggest the creation of Advisory Committees with each of these posts under the Government of India. For instance at the Imperial Agricultural Research Institute, New Delhi, an advisory committee with the Imperial Botanist as the convener and another with the Imperial Mycologist as the convener may be formed. Not more than 5 or 6 members should be on each committee and the I.B.S. and workers of the Universities will be represented on these committees.

A great deal of valuable research work on Cytology is being done in the Universities, but the time has come to consider seriously whether a little more utilitarian bias would not be all to the good. The Imperial Botanist is breeding new crops and is also in touch with the Government workers in the Provinces doing similar work. The breeder, we know, must have the guidance of the cytologist whose microscopical analysis will indicate the number of chromosomes in the cells of the various parent plants, and the general behaviour between the chromosomes to be mated in the distant crossing. It is not possible either for the Imperial Botanist or the Provincial Agricultural Botanist to study the cytogenetics of all the plants raised. The advisory committee will see that extra material is distributed to the workers in the Universities and private research institutes. They will advise and arrange for new lines of work and work of All-India importance. Whenever team work is required in which workers from different parts of India will be needed, instead of limiting the choice of workers to the agricultural departments only, they could arrange for such team work by taking workers from every sphere and recommend financial help if necessary. The same may be said about the Imperial Mycologist's department. Much systematic work on Indian Fungi yet remains to be done. An idea of this may be had if we look at the Supplement I to Fungi of India in which out of a little over 500 species listed, very nearly 300 have been recorded and published by workers in my laboratory, and I cannot say we have touched even the fringe of the work that remains yet to be done from the Panjab. The Committee, if appointed, could easily arrange for working tables for research workers, who could be sent to the Agricultural Research Institute at Delhi to compare and complete this systematic work. Provision for two tables which could be reserved for 15 days at a time could easily be made, so that annually 24 people could work there.* We want more co-operation, and the work has to be co-ordinated.

At one time, there used to be held at Pusa, a conference of mycological workers from all parts of India in which both Government and non-Government people participated. This conference should be revived. At such conferences results of the work done

* I am thankful to Dr. Padwick, the Imperial Mycologist, for the information that this is already in force and that research workers from all parts of the country are given the best available facilities for work there.

at different centres could be correlated and plans for the following year prepared.

The Botanist and the Mycologist at the Forest Research Institute at Dehra Dun, could also enlarge their sphere of work by establishing contact with workers in the Universities. I am not trying to belittle the very useful and important contributions made by the Forest Botanist, specially on Forest Ecology and by the Mycologist, specially in establishing the alternate hosts of rusts of the forest plants and thereby removing many obscurities. We are very grateful to them. But their departments are either one or two-men departments, and certainly much more work could be done by properly harnessing the resources, both in men and materials, in the Universities. While talking of the Forest Research Institute, I must also pay tribute to the Wood Technologist there for the excellent and important work now being done by him.

The Indian Botanical Survey is now practically defunct, but I see no reason why survey work should not continue and that better than before if carried on a regional basis. We have now in the Universities and Colleges men trained in this country and abroad, who could take up the systematic study of the higher plants with credit. A certain amount of financial help will have to be given in some cases and herbarium sheets will have to be loaned out; otherwise facilities have to be given to the workers to work in the Herbarium of the Royal Botanic Garden, without any interference. This work will be arranged by the Garden Advisory Committee, who will distribute the work on a regional basis to trained workers in different parts of the country. If this is done, we shall have not only a complete revised flora of India on regional basis but also the Herbarium will be very much enriched. In this way a National Herbarium for India as envisaged by Prof. S. P. Agharkar in his opening remarks on a discussion on this subject in the Science Congress Jubilee Session could be built up. All this will be done at a very nominal expense by proper co-operation, collaboration and co-ordination. Our country is like a vast continent. There are room and need for many more workers. The Advisory Committee on which the I.B.S. will be represented will also see that whole-time men in the Herbarium devote their energy to revision of families and study of new plants belonging to the higher groups of plants for which the herbarium is reputed and not dissipate their energy by dabbling in every branch of plant-life. After all we have a very limited number of men in these departments, and they must fully utilize the facilities afforded them. Here I must acknowledge the important work done by the new Curator of the Herbarium in bringing out "A Revision of the *Labiatae* of the Indian Empire". This is an important contribution, and there is need for such revision of other families and genera. The Royal Botanic Garden Herbarium is primarily a herbarium for higher plants and the vast material there will provide work for scores of systematic workers for many years to come. Any attempt to make it a repository for all kinds of plants

would be futile and should be deprecated. If I have a polypore to name, I shall send the same to Professor S. R. Bose at the Carmichael Medical College, Calcutta. I suppose others in this country will do the same. Similarly perhaps if any Himalayan Liverwort has to be compared and named, it will be sent to the Panjab University Botanical Herbarium as is done now from all quarters. As long as described plants are kept in properly looked after herbariums, and are available for consultation why should one worry which herbarium it is? If the principle of co-operation, and if necessary, collaboration with University workers which I am advocating had been followed, a new edition of Prain's Bengal Plants would have seen the light many years back: It is a pity that no up-to-date flora should be available to the Bengal Botanists.

The officer-in-charge of the Industrial Section of the Botanical Survey of India has brought out useful catalogues of Medicinal Plant Exhibits and of Spice and Fodder Plant Exhibits in the Industrial Section of the Indian Museum. He has also published useful handy notes regarding a number of Indian Medicinal plants. But unfortunately his is also practically a one-man department. If there were an advisory committee here too, more work could be arranged on co-operative basis and the whole co-ordinated. The Imperial Sugarcane Research Station at Coimbatore is no doubt doing valuable work, but the same suggestions as I have made in the case of the Imperial Botanist are applicable here. The recent decision to add a sugarcane physiologist on the staff there is a welcome one.

Advisory Committees are very helpful for proper planning and co-ordination. The Indian Botanical Society must press for the appointment of advisory committees. The Director of the newly created Scientific and Industrial Research Bureau has already shown how much more work could be done, if specialist sub-committees were appointed for advisory purposes and for giving directions. The Director has already encouraged many research schemes in collaboration with the University and while making a "Compilation of Annual List of Industrial Researches in India" he is taking into account the researches carried out or in progress at the various University and College laboratories which had not been done before. "The Indian Central Jute Committee in its meeting last September decided on the policy of collaboration with the Universities of Calcutta and Dacca and to co-opt some professors of these Universities on its technological and agricultural sub-committees. The immediate objects of collaboration were stated to be primarily twofold. *First* the committee thought that the University scientists, many of whom were perhaps working on similar lines, might offer valuable advice on the work that was being done in the Committee's Research sections. Even if this immediate work might be different from the investigations that were being undertaken by the different technical sections of the committee, they felt that their familiarity with the basic scientific methods and processes might be of considerable help and value to the committee's

research workers. *Secondly*, the Committee were inclined to think that the Universities, on their part, could also further their aims and objects by undertaking fundamental research on a number of subjects for which there was not, and indeed could not be, any room in the programmes of work laid down for the different sections of the committee. Such fundamental investigation might lead to results of far-reaching consequence which might be of abiding benefit to the jute industry of this country." I have quoted the above from a Press report of the meeting, as it appeared to me like a very bright silver lining in the black cloud of mistrust and isolationism. It is very encouraging indeed. I do hope the President of the Central Cotton Committee who is the Vice-Chairman of the I.C.A.R. will bring his liberal and healthy outlook to the affairs of the latter body as well, and give the University workers their rightful place in the work for the good of the country. The same policy should be followed in cotton, wheat and tea research schemes. Among other Government departments, the Indian Geological Survey has been co-operating with Prof. Sahni by sending out Museum specimens and in other ways, and I am happy to find that Prof. Sahni from the University, is co-ordinating the palaeobotanical research in India.

The proposal for starting a Marine Biological Station at Krussadi, I remember, was discussed at a meeting of the I.B.S. Last year the Indian Science Congress took up the matter and a committee has since then been appointed which will discuss the proposals received at the present session of the Science Congress. The proposal is to start the first station at the Krussadi Island. A local committee appointed for this purpose that met at Madras thought that to make a useful start, a non-recurring expenditure of Rs. 40,000 and a recurring one of Rs. 5,000 was required. If these sums are available, well and good, but I think much useful work on many aspects of marine-biology could be done if only facilities for occupying existing accommodation were obtained. For us in the Punjab, the nearest coast is that at Karachi, and we have been fortunate in past years in getting permission to occupy the military huts at Manora Island at Karachi, and much useful work has been done by the Zoologists and Botanists on Marine Fauna and Flora. We have very recently brought out Part I of the Marine Algæ from Karachi in which the ecological study of the plants has not been neglected. Similarly for the study of the plants of higher altitude temporary hill-laboratories for a fortnight or three weeks could be arranged in which two or more Universities could participate. We arranged one such hill-laboratory last summer for about a fortnight at Narkanda in the Punjab hills for the special study of forest ecology besides collection of plants and the study of the same on the spot. Lectures and laboratory work were arranged for the students.

A summer hill school with about seven or eight teachers from two or more Universities and some twenty senior students is quite

a practical proposition, and much useful work could be done in a short time.

I shall not tax your patience for long but before I close I shall appeal, specially to the University teachers who are in charge of higher teaching and research, to give an applied bias to our subject. We have to encourage researches of an utilitarian nature. The present world condition demands it. If this is done, our students will then be more familiar with the problems of our country for the solution of which a knowledge of Botany is required. They will also find the study of the subject much more interesting. Our teachers have to study the needs of the various industrial and commercial concerns in which they could make their knowledge useful. Emphasis will have to be laid on the study of drug, and fibre yielding plants and other plants capable of yielding tannins, dyes and other valuable products. In the Punjab, we have a valuable sports goods industry. These concerns carry on quite a decent export trade. Many species of *Morus* are used for this purpose. Botanists (Breeders and Cytologists) could study the properties most sought for, and could combine these by breeding suitable species. We have in our Botanic Garden at Lahore raised new plants by crossing the local species of *Ephedra* with the Chinese and Himalayan species of the plant with a view to producing a species for local cultivation, rich in ephedrin. When the plants have grown a little more, analysis for the ephedrin content will be made. Plant poisons are more and more being utilised as insecticides, and our students should be familiar with this aspect. They must know more about the food, spice and fodder plants. Study of diseases and disease control methods should form a part of the higher study of Botany. Micro-biology should have a better recognition. Researches on many aspects of Plant Physiology could be carried on in the University without much difficulty; for example, study of some aspects of vernalization, effect of growth hormones, inducement of polyploidy by colchicine and other chemicals, work on hydroponics with a view to commercial exploitation and so on. Experiments with excised roots to find the effect of various nutrients could be made on a laboratory table. Students have to be taken to the fields, sea-side and hills more often so that they may become familiar with those plants. They should know our forests better, know also the effects of afforestation and deforestation. They must learn how to explore for new plants. Search for the wild species is an extremely important work. "In plant research, we should aim at mobilizing the plant resources of the world for human uses. The Soviet philosophy in no way regards the botanist as an amateur of plants, but as a person whose work is concerned with one of the bases of civilization. The resources of plant life have to be utilized for the material and intellectual benefit of humanity" (Soviet Science). The methods of utilizing the plant life and energy of tropical and sub-tropical countries have not yet been worked out. We should strive for the same.

I have made a very cursory survey just to illustrate my point. I have emphasized the utilitarian application of Botany. This, however, may only be borne in mind and should not be the motive of research; otherwise fundamental and far-reaching results will never be achieved. The Universities, specially, should be free from narrow commercial motives. "Furthermore, the research worker need have no fear that his theoretical results will not find a useful application, for theoretical science and applied science are inseparable; they are one and the same, as the fruit is to the tree" (Seifriz).

Now I have come to the end of my Address. Many of you perhaps hold views different from mine. I might have offended some of you who are present here to-day, or will perhaps offend more people outside who may read my address later. But believe me, I have said what I felt sincerely and what I considered must be said. I assure you, however, I bear no malice to anyone. I thank you sincerely for giving me this opportunity to express my views. I again thank you for the patient hearing.

ON THE LIFE-HISTORY AND CYTOLOGY OF
MICRODICTYON TENUIUS (AG.) DECSNE.*

Sikorula, Caloniaceae

(PRELIMINARY NOTE)

BY M. O. P. IYENGAR, M.A., PH.D. (LOND.), F.I.S.

AND

K. R. RAMANATHAN, B.SC. (HONS.), M.SC. (MADRAS)

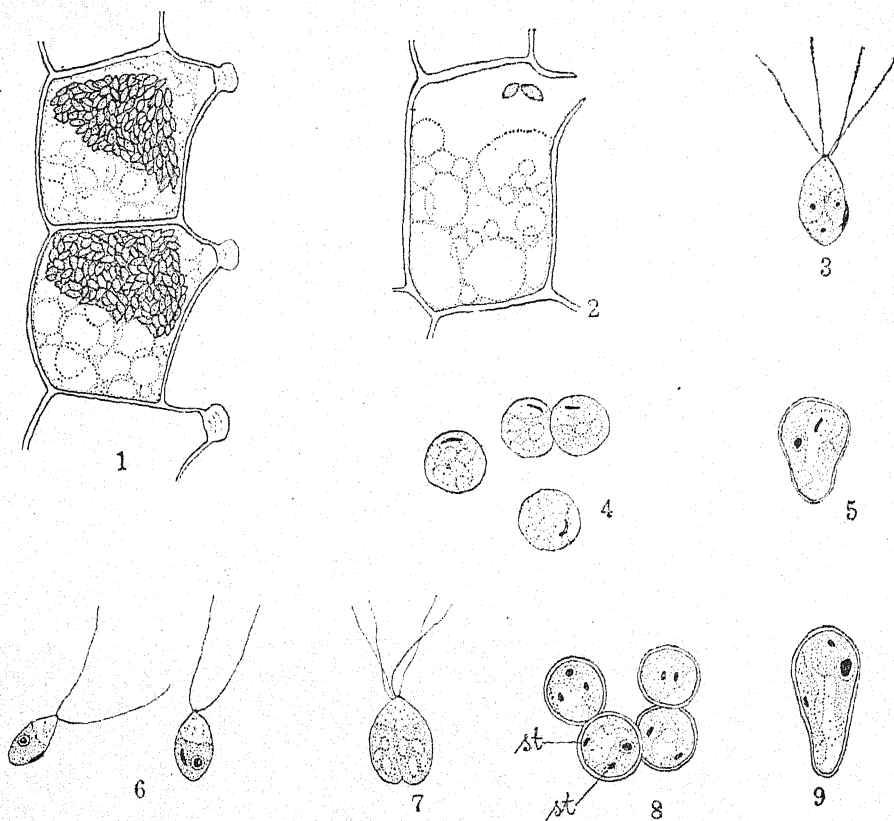
Received for publication on January 25, 1941

VERY little is known regarding the reproduction in the genus *Microdictyon*. Wille (1897, p. 151) states that swarmspores may be formed from all the cells of the thallus, but does not give any details or figures. Most of the authors after him (Collins, 1909; Printz, 1927; Setchell, 1929) who refer to the reproduction in this genus do not add any further information. Boergesen (1925) is the only author who has given some details regarding the reproduction in this genus. He found in *Microdictyon Calodictyon* (Mont.) Decsne. from the Canary Islands, that some of the cells of the thallus were transformed into zoosporangia with short conical projections, having apical openings for the escape of the swarmers. He described the swarmers as zoospores, but did not observe actually their escape from the cells, nor did he mention the number of cilia in the swarmers. His figures also show only a number of rounded bodies without any cilia, presumably swarmers, inside the opened sporangia. Thus the exact nature of the swarmers, whether they were biciliate or quadriciliate and whether they were gametes or zoospores is still unknown.

The authors collected recently at Rameswaram in South India a species of *Microdictyon*, coming near *Microdictyon tenuius* (Ag.) Decsne. and brought the alga in a living condition to Madras and followed its life-history in laboratory cultures. The plants in the cultures produced plenty of swarmers on a number of days. The swarmers were formed in any cell of the thallus. The swarmers when fully formed became compacted into a dark mass in one portion of the cell, leaving the remaining portion of the cell cavity occupied by a refractive frothy mucilage (Pl. III, Figs. 1 and 2; Text-fig. 1). The swarmers escaped outside through a conical opening (Text-figs. 1 and 2) similar to that observed by Boergesen (1925) in *Microdictyon Calodictyon*.

The plants forming swarmers were of two types, one set of plants forming only biciliate gametes (Text-fig. 6) and the other forming only quadriciliate zoospores (Text-fig. 3). The two sets of plants were quite similar to one another in their external

* From the University Botany Laboratory, Madras.



Text-figs. 1-9. *Microdictyon tenuius* (Ag.) Decsne. Fig. 1. Two cells with fully formed swimmers ready for release: note the swimmers compacted into a dark mass toward one portion of the cell and the remaining portion of the cell-cavity filled with frothy, refractive mucilage. Fig. 2. A sporangial cell after the escape of the swimmers, showing the frothy mucilage and two swimmers left behind. Fig. 3. A quadriciliate zoospore. Fig. 4. Zoospores just come to rest. Fig. 5. Zoospore germling 24 hours old. Fig. 6. Biciliated gametes. Fig. 7. Conjugation of two gametes. Fig. 8. Zygotes surrounded with a wall and showing two eyespots in each. Fig. 9. Zygotic germling 24 hours old. *st*; eyespot (Figs. 1 & 2 $\times 370$; rest $\times 1070$).

appearance. The quadriciliate swimmers after swimming for some time settled down and grew into small germlings (Text-figs. 4, 5). The biciliate swimmers, on the other hand, kept swimming for a long time and finally degenerated and died. No fusion was observed between the biciliate swimmers produced from any single thallus. But in some of the cultures where a number of different thalli were kept together, a large number of zygotes were seen, each one having two eyespots, suggesting clearly that they were the result of fusion of the swimmers from the different thalli (Text-fig. 8; Pl. III, Fig. 3). In one instance a late stage in the

conjugation of the two biciliated gametes was observed where the fusion of the protoplasts was not yet complete (Text-fig. 7). The fact that the gametes from the same plant did not conjugate but degenerated and that plenty of zygotes were formed in cultures in which a number of different thalli were placed together suggests that the sexual plants are evidently dioecious. The zygotes after surrounding themselves with a wall germinated immediately and formed small germings quite similar to those formed by the zoospores (Text-fig. 9).

A cytological study of the alga showed that there are two types of plants, one haploid having 16-18 chromosomes and the other diploid having 32-36 chromosomes. Reduction division was observed in the diploid plant just before swarmer formation. Typical synizetic and diakinetik stages (Pl. III, Figs. 4, 5) were observed and 16-18 bivalents were counted during diakinesis.

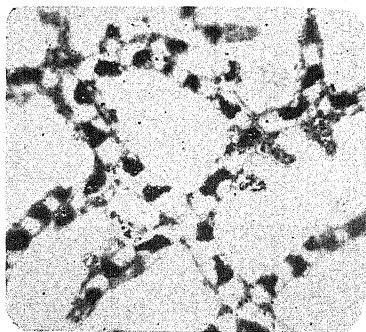
The facts detailed above clearly point to the existence of an alternation of a haploid sexual with a diploid asexual generation in *Microdictyon tenuius* (Ag.) Decsne., quite similar to what is seen in the Cladophoraceæ and Ulvaceæ and also in the allied genus *Anadyomene* (Iyengar and Ramanathan, 1940).

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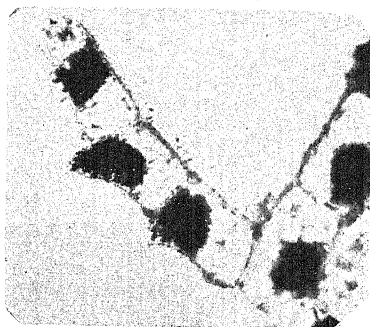
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EXPLANATION OF PLATE III

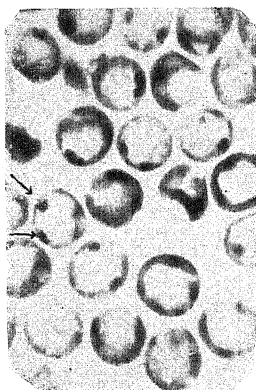
- FIG. 1. Thallus with practically all the cells forming swarmers. $\times 45$.
- FIG. 2. A portion of the above enlarged; note the swarmers compacted into a dark mass towards one portion of the cell and the remaining portion of the cell-cavity filled with a frothy, refractive mucilage. $\times 132$.
- FIG. 3. Zygotes, with the two eyespots clearly seen in some of them; (the arrows point to the two eyespots in one of the zygotes). $\times 1250$.
- FIGS. 4 & 5. Synaptic and diakinetik stages respectively of the reduction division. (Fig. 4 $\times 1875$, Fig. 5 $\times 1460$).



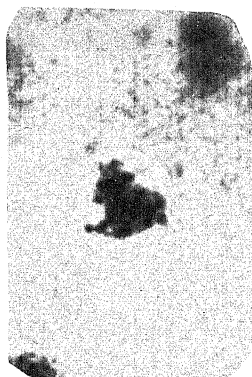
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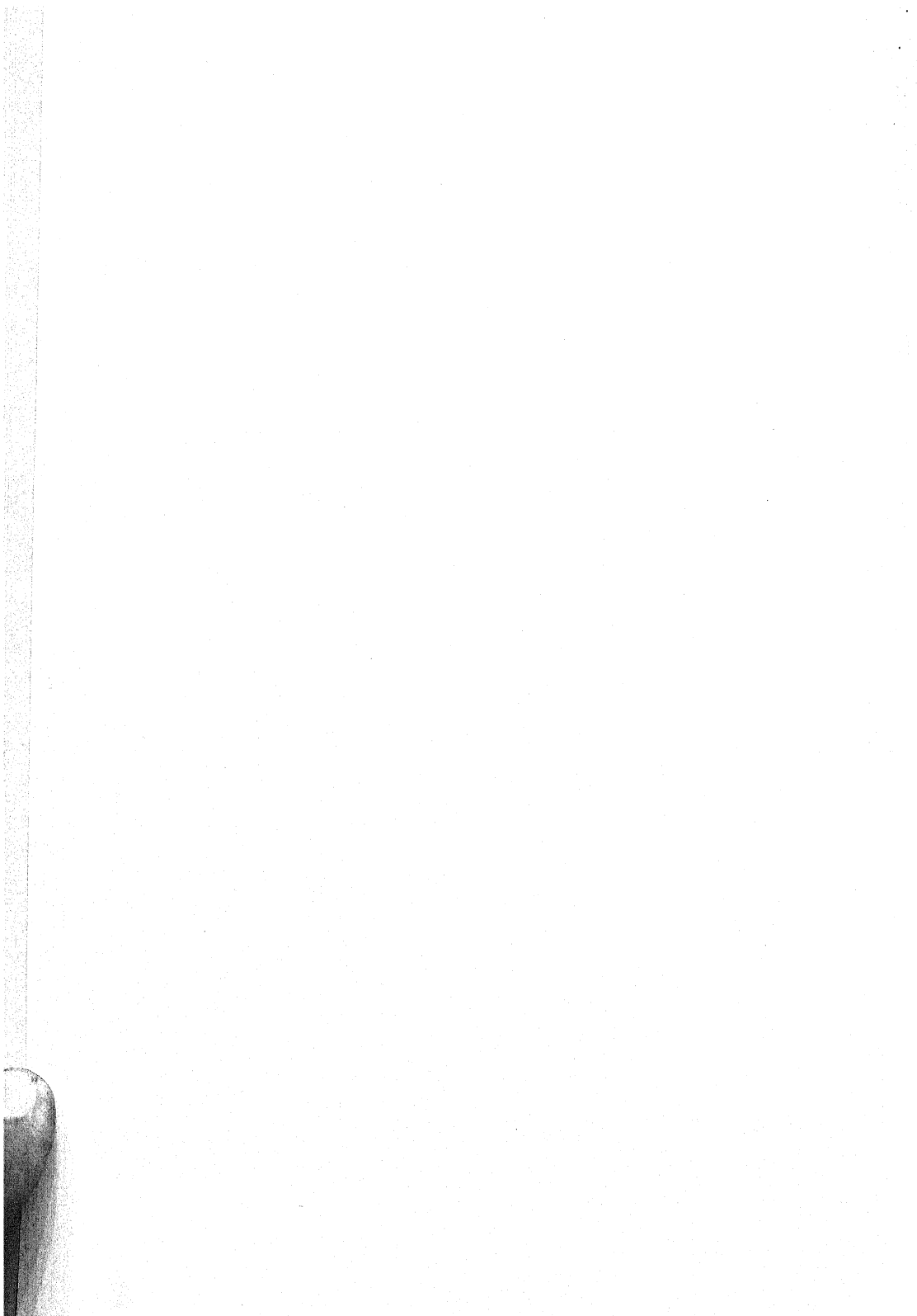
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AND
K. R. RAMANATHAN

MICRODICTYON TENUIUS (AG.) DECSNE.



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THE SLIDING, GLIDING, SYMPLASTIC OR THE INTRUSIVE GROWTH OF THE CAMBIUM CELLS AND THEIR DERIVATIVES IN HIGHER VASCULAR PLANTS*

BY GIRIJA P. MAJUMDAR, M.Sc., Ph.D.

Botanical Laboratory, Presidency College, Calcutta

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ALL the elements of the permanent tissues of a vascular plant are derived from the eumeristem, cells of which are more or less uniform so far as their size and structure are concerned. During their growth and differentiation the cells vary both in size and character. What are the developmental relations of the various elements and how are intercellular re-adjustments effected during the transitional stages, are problems which have not yet been satisfactorily answered. In these pages re-adjustment of the cambial cells and also of their derivatives in higher vascular plants have been discussed.

In plants which grow in thickness and in which the elongation of the axis as a whole has ceased the cambium cylinder extends peripherally from year to year; the fibre cells, particularly the secondary fibres, grow many times the length of the initials from which they become differentiated. How does this happen?

Bailey (1923) calculated that in 60 years the peripheral extension of the cambial ring in *Pinus strobus* amounted to sixty-fold, and during this period the fusiform initials were seen to grow in number from 724 to some 23,100, and in their length they grew about five times their original length. He further calculated that one-fifth of this number was the result of cell division, the rest were due to the increase in the length of the initials themselves. From this data Priestley (1930) worked out that in *Pinus strobus* the fusiform initials divided once in fifteen years!

* Paper read before the Botany Section at the Benares Meeting of the Indian Science Congress Association, January, 1941.

Cambial Division in Conifers and in Less Specialised Dicotyledons (Non-stratified).—Nägeli (1868) suggested that the tangential increase in the number of cells in the cambial cylinder might be effected by their radial longitudinal divisions. But later investigators, viz., Robert Hartig (1895), Klinken (1914), Bailey (1923), from their extensive studies showed that the normal divisions of cambial cells in all Gymnosperms is by transverse or by pseudo-transverse walls, and the increase in the periphery of cambium in these plants is primarily due to the elongation and adjustment of transversely dividing fusiform initials.

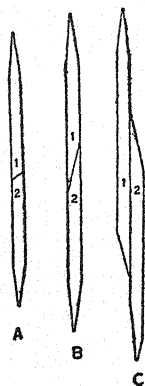
Neeff (1920) working on *Tilia tomentosa* found no distinction in this respect between the lateral meristems of Dicotyledons and Gymnosperms. Bailey observed in *Drimys* and *Trochodendron*, which are vessel-less Dicotyledons, that the type of cambium division conformed strictly to the Conifer type, i.e., by transverse or pseudo-transverse walls.

Cambial Division in Highly Specialised Dicotyledons (Stratified).—It is only in Dicotyledons with stratified cambia that radial longitudinal divisions of cambium cells have been observed, so far as I know, by Bailey (1923), Kleinmann (1923), Beijer (1927) and by me in *Heracleum Sphondylium* (1940, Fig. 6). But stratified cambia are very rare in Dicotyledons. Moll and Janssonius (1906-26) in a census of the Javanese woods found only about 5% with stratified cambia.

When such is the position it is very difficult to visualize how these elongating cells in the cambia of Conifers and less specialised Dicotyledons, adjust their position so that they come to lie side by side in the peripherally extending cambial ring. The same difficulty arises in the case of elongating fibre-mother-cells in higher Dicotyledons.

The following three theories have been advanced as a solution to this apparently difficult problem. They are (1) the theory of the gliding, or sliding growth (Krabbe, Bailey), (2) symplastic (Priestley), and (3) intrusive (Sinnott and Bloch) growth movement of the cambial initials or the fibre-mother-cells.

The Sliding or Gliding Growth Movement.—Krabbe (1886 ; Scott, 1888-89) was the first to suggest the sliding or gliding growth (gleitende Wachstum) of the cambial cells as a solution to this problem. This has been later elaborated by Bailey (1923) and incorporated by Eames and MacDaniels (1925) in their text-book. According to Bailey during development the fusiform initials in a non-stratified cambium "elongate sliding by one another until they attain a certain size. They then divide by means of a more or less oblique partition into short halves which in turn elongate and divide" (Fig. 1, A, B, C). He says the products of these divisions elongate and crowd by one another resulting in an increase in the girth of the cambium. Bailey measured the elongation of cambial initials themselves in *Pinus strobus* about five times at the



Text-fig. 1. Division of a fusiform initial by pseudo-transverse wall; daughter cells, 1, 2, elongate and slide by one another as they grow. From Eames & MacDaniels (after Bailey), p. 147.

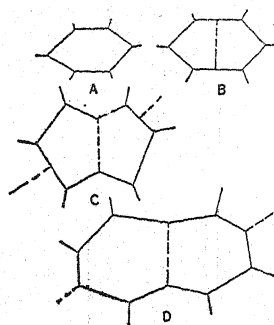
end of the fifty-ninth year. Kundu (1939) noticed hemp and jute fibres to elongate three times their cambium initials.

All these involve a considerable sliding past one another, and it is very difficult to comprehend this relative movement under the conditions existing in the developing and differentiating tissues, and also in the absence of definite evidence.

The Symplastic Growth Movement.—Tammes (1913; Aldaba, 1927) raised objection to the sliding growth movement as proposed by Krabbe. He observed that while the lower end of a fibre wall had been undergoing thickening and held firmly amongst fully differentiated elements the upper, in an internode which had not yet ceased to grow in length, was still elongating under compression.

As an alternative to sliding growth movement Priestley (1930) elaborated his symplastic growth movement on the basis of what actually happens in the apical meristem during cell adjustment following each division. Fig. 2, A, B, C show progressive changes in cell shape in which the new position in C can be reached without any slip between the walls of the dividing cell and its neighbours. The whole wall grows and stretches as a common 'threeply' membrane as Priestley would describe the stage (*cf.* D'Arcy W. Thompson on Growth and Form, VII, 1917). He concluded "that the adjustment of cell shape and cell position takes place as a result of a gradual *mutual* adjustment of position in which the partition walls adjust their tension and position as a common framework without the necessity for any slip between any two cellulose walls facing one another across a common middle lamella" (p. 102).

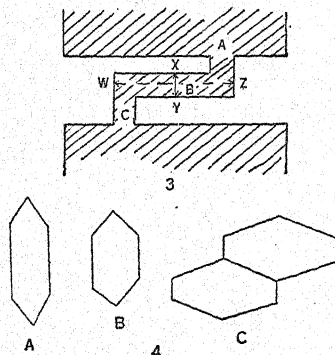
This adjustment due to symplastic growth, however, assumes that the protoplasts are in a semifluid condition and are separated from one another by extremely plastic walls.



Text-fig. 2. A-D, adjustment of cell shape and cell position in the apical meristem after each cell division (after Priestley, p. 101).

Both Kleinmann (1923) and Beijer (1927) observed that following each cell division in a cambial cell the readjustment of these plastic cells takes place exactly in the manner described for the apical meristem (see Priestley's figs. 3-7, pp. 104, 105).

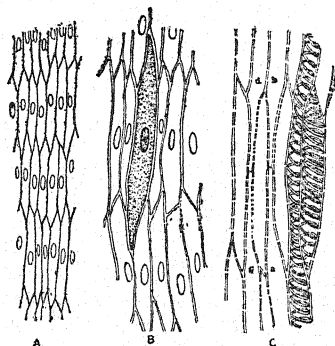
But the experimental evidence supplied by Teodorosco and Popesco (1915) appears very conclusive on the readjustment of cells by symplastic growth movement. Fig. 3 shows the strip A B C left of the bark and other tissues while from the rest of the axis the bark has been removed after the manner suggested by Czapek. These two investigators showed that axes of new xylem and phloem in the regions A and C were vertical, while those in B, horizontal. They showed further that the cambial cells in B became first isodiametrical, then they elongated symplastically horizontally (Fig. 4, A, B, C). Bailey and Miss Tupper Carey (1930) noticed the deformation of plastic walls under pressure.



Text-figs. 3 and 4. Illustrating the experiments of Teodorosco and Popesco : the ring experiment and readjustment of cambium cells with renewed growth (from Priestley, pp. 135 and 136).

Priestley worked out and showed that the radial pressure caused by the differentiating and maturing tracheids in Conifers, and the lateral pressure of the horizontally expanding and differentiating xylem vessels in Dicotyledons might cause sufficient deformation resulting in the longitudinal extension of the cambium initials to account for the increase in the girth of the cambium cylinder, and the longitudinal elongation of the cambium and the fibre-mother-cells. But when the vessels are differentiating do the fibre-mother-cells remain sufficiently plastic to account for the enormous increase in the length of adult fibres?

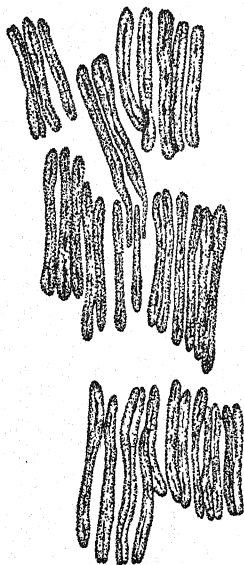
The Intrusive Growth Movement.—Sinrott and Bloch (1939) suggested that the so-called sliding growth is really intrusive growth due to localised active growth confined to the two ends of a cambium initial, or of a fibre-mother-cell. Fig. 5, A, B, C (Pl. Figs. 1, 2, 3) shows cambial cells in three stages of growth and differentiation in a species of *Laburnum*. Fig. 5 A shows cambium initials in the



Text-fig. 5 A, B, C. Camera lucida drawings of the stratified cambium from cambium strips of *Laburnum* sp. showing growth, elongation and differentiation of a fibre from cambium initials (A $\times 81$; B, C $\times 135$).

stratified stage; Fig. 5 B, one of the cambium cells differentiating into a fibre. The cell is growing by advancing tips between the cambium cells of the upper and the lower series, the secondary thickening has not yet started. Fig. 5 C shows the final stage where the fibre-mother-cells of the upper and lower series (*a, b*; *c, d*) have already differentiated into fibres with their tapering ends meeting midway in the middle series.

Fig. 6 (Pl. Figs. 4 and 5) is the stratified cambium initials from the growing apex of *Heracleum Sphondylium*. The slide has been prepared from macerated material previously fixed in chrom-acetic-acid solution. Here the cambium initials are seen in different lengths, some just split radially longitudinally, and in one place two initials from an upper series intruded by their ends between initials of the lower series. Plate Fig. 6 shows a patch of secondary



Text-fig. 6. Camera lucida drawing of the macerated cambium from the growing apex of *Heracleum* showing radial longitudinal divisions and intrusive growth of the initials ($\times 622$).

fibres in tranverse section. It will be noticed that between radial rows of fibres small fibre ends are present occupying the inter-cellular space areas between the former. Evidently these ends of the fibres intruded between original rows of fibres during their growth and differentiation.

DISCUSSION

There have been three different suggestions to explain the increase in the peripheral extension of the cambial cylinder during the secondary growth in thickness of the stems of Conifers and of the less specialised Dicotyledons, and incidentally also to explain the increase in length of the fibre-mother-cells in the wood and phloem in the axes of the Dicotyledons which have already ceased to extend in length.

It appears that no distinction has been made between the cambium and the fibre-mother initials. Cambial cells are characterised by frequent divisions whereas the fibre-mother-cells are the non-meristematic growing and differentiating cells derived from the last division of the cambium initials. After the last division of the cambium cells when the daughter cells are differentiating into fibres or tracheids they should be regarded according to their final form either fibre- or tracheid-mother-cells.

In Conifers the tracheids are seldom much longer than the fusiform initials from which they are directly derived. The fully

developed fusiform initials and their derivatives, the tracheids, are practically the two stages of the same individual. Whereas in the highly specialised Dicotyledons the fibres become several times the length of the fibre-mother-cells during differentiation.

There exists, therefore, a fundamental difference between the increase in length and adjustment of tracheid and fibre-mother-cells in Conifers and in the highly specialised Dicotyledons respectively. In the first case, *i.e.*, in Conifers and also in the less specialised Dicotyledons cell readjustment takes place in a more or less plastic system where all the cells are elongating equally under a uniform radial pressure. Here the cell adjustment is undoubtedly brought about by a symplastic growth movement as visualized by Priestley. But in higher Dicotyledons the question appears different. Here the fibre-mother-cells elongate many times the cambium initials, in a differentiating tissue which is heterogeneous in composition and in which the cells do not seem to be in a very plastic condition. The adjustment appears to take place by the intrusive growth movement confined primarily to the two ends of the fibre-mother-cells.

If we are to understand by gliding or sliding movement wholesale slip of one cell along the faces of its neighbours there is no direct evidence to prove it; but if on the other hand, the progress of the extending cell between its neighbours is meant to be effected by localised active growth of its ends, the whole process is not only explainable but also is consistent with observed facts. This mode of growth of a cell by one or both the ends has been called by Sinnott and Bloch (1939) *intrusive growth*.

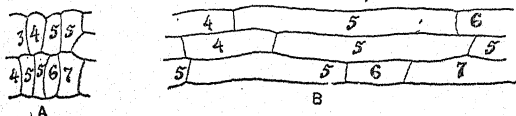
Three objections might be raised against the acceptance of this mode of growth of the differentiating cambium or fibre initials, *viz.*, (1) when a cell intrudes between two cells the protoplasmic connections (plasmodesma) between the latter are ruptured. At this stage, however, the cells are in the primary condition. Strasburger (1901) and Sharp (1926) state that protoplasmic connections, if severed, might secondarily be re-established.

(2) In such conditions halves of a pit formation should not correspond with each other. But pit formation takes place only during the progress of secondary thickening. Kundu (1939) while studying the development of the fibres of jute and hemp found that secondary thickening and consequently pit formation, started only after the fibres had attained their full elongation. As a matter of fact this method of secondary thickening distinguishes a fibre from a collenchyma cell where elongation and secondary thickening proceed simultaneously (Majumdar, 1940).

(3) The third objection is against the conception of the differential growth of the cell-wall or the cell. The intrusive growth is based on this conception. Is it a new conception?

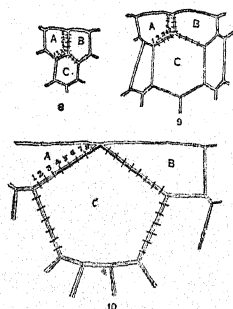
Growth of a cell near the tip is a normal feature in root hair cells, in callus and tyloses cells, in certain idioblasts and in the

latex ducts of the non-articulate type. Klinken (1914), Neeff (1914), Bailey (1923), Bailey and Kerr (1934) observed apically localised growth of cambial cells; Zander (1928) and Schaffstein (1932) noted growth near the tips of latex ducts in regions where other tissues of the plant are still growing. Sinnott and Bloch (1939) saw differential cell growth in the surface layers of the roots of *Poa trivialis*, *Phleum pratense*, and other species (Fig. 7, A, B).



Text-fig. 7. *Phleum pratense*: A, B showing low the lower wall of cell 5 of upper row in A shows differential growth in contact with cells 5, 6 and 7 in the lower row in B. From Sinnott & Bloch, p. 623.

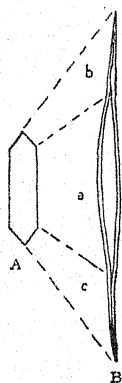
Rothert (1900) and Guttenberg (1902) observed localised growth in crystal cells (Figs. 8, 9, 10) and Knoll (1905) found the same



Text-figs. 8, 9 and 10. Diagrammatic representation of the intrusive growth of a crystal cell of the leaf of *Citrus* after von Guttenberg. From Sinnott & Bloch, p. 633.

thing in hair cells. The writer saw actual progress of the apices of a tracheid mother cell in *Laburnum* sp., and of the cambium initials in *Heracleum*. Therefore the differential growth of a cell or its wall cannot be regarded as a new conception.

The fibres in Dicotyledons are more or less spindle-shaped, a swollen middle portion with two ends long tapering. The middle portion corresponds to the body of the fibre-mother-cell and the long tapering ends, its intrusively grown apices (Fig. 11 A, B). Kundu (1939) saw the same thing in the fibres of jute and hemp. This has also been observed in *Laburnum* (Fig. 5 C; Pl. Fig. 3) and in *Heracleum*. A comparison between the adult fibre cell and its mother reveals that during differentiation not only the latter has grown in length by the two ends, but the middle region has also grown apparently in length and volume (Fig. 11 A, B).



Text-fig. 11. Diagrammatic representation of the differentiation of a fibre from its mother cell; relative growth of its different regions a, b and c, during elongation is shown.

While examining a sample of previously fixed and then macerated collenchyma initials of Rhubarb petiole, stained in 1% aqueous eosin solution, the present writer observed a very interesting stage. In each elongating collenchyma cell three dense zones in the protoplast were noticed, *viz.*, in the middle of the cell with the nucleus embedded in it, and at the two ends without nucleus, the rest of the cytoplasm was less dense and almost colourless. This suggested to the author that active growth in length and volume of an elongating and differentiating fibre cell might be confined to its two ends and the middle region.

In the intrusive growth the tip advances through the neighbouring cells. How is then the advance between the neighbouring cells effected? Neeff (1914) presented evidence to show that the sharp expanding cell tips digest the cementing substance between two cells (*cf.* tips of latex cells), rupture protoplasmic connections and grows between the two cells. Eames and MacDaniels (1925) also suggest that the growth takes place at the tips only, and the dissolution of the middle lamella of the cells on either side is due to some enzyme action, or may be due to growth processes (p. 150). It is difficult to say if mechanical pressure caused by the forward movement of the growing tips through the slimy intercellular pectic substances, has anything to do with the splitting of the common wall. This will, however, form the subject-matter of a different paper.

SUMMARY

In stems which grow in thickness and which have definitely ceased to elongate the cambium cylinder extends peripherally from year to year.

The fibres, particularly the phloem and xylem fibres during their differentiation in a heterogeneous tissue, grow many times the length of their mother cells, the cambium derivatives.

Extensive studies on cambium and its activities have shown that in Conifers and Dicotyledons which have non-stratified cambia, the increase in the periphery of cambium is effected by the elongation and adjustment of the daughter cells derived from the transverse divisions of the fusiform initials; and it is only in the highly specialised Dicotyledons with stratified cambia that the peripheral extension is maintained by the radial longitudinal divisions of the cambial cells, but stratified cambia are very rare in Dicotyledons.

So far the following three suggestions have been made to explain how the intercellular readjustments are brought about, *viz.*,

1. By the sliding or gliding growth movement which results in the slip of a cell along the faces of its neighbours;
2. By the symplastic growth movement in which the adjustment of the cell shape and cell position is brought about by a pressure on the plastic cambial system; and
3. By the intrusive growth movement in which the progress of the extending cell between its neighbours is effected by the active growth of its ends. This may be taken as a modification of the first.

There is no direct evidence to show that a cell slips along the faces of its neighbours during elongation and differentiation, but readjustment by the symplastic growth movement without any slip, following each cell division, in the apical meristem is easily understandable. This is also possible in a system, such as the cambium, which is not only plastic but is also differentiating under a constant pressure. Teodorosco and Popesco have already demonstrated such a possibility in the cambium, but it is doubtful if the fibre-mother-cells elongate in such a system.

In higher and highly specialised Dicotyledons the elongation of the fibres during differentiation appears to take place by the intrusive growth of their ends between their neighbours. This is not only explicable but is also based on evidence produced in this paper.

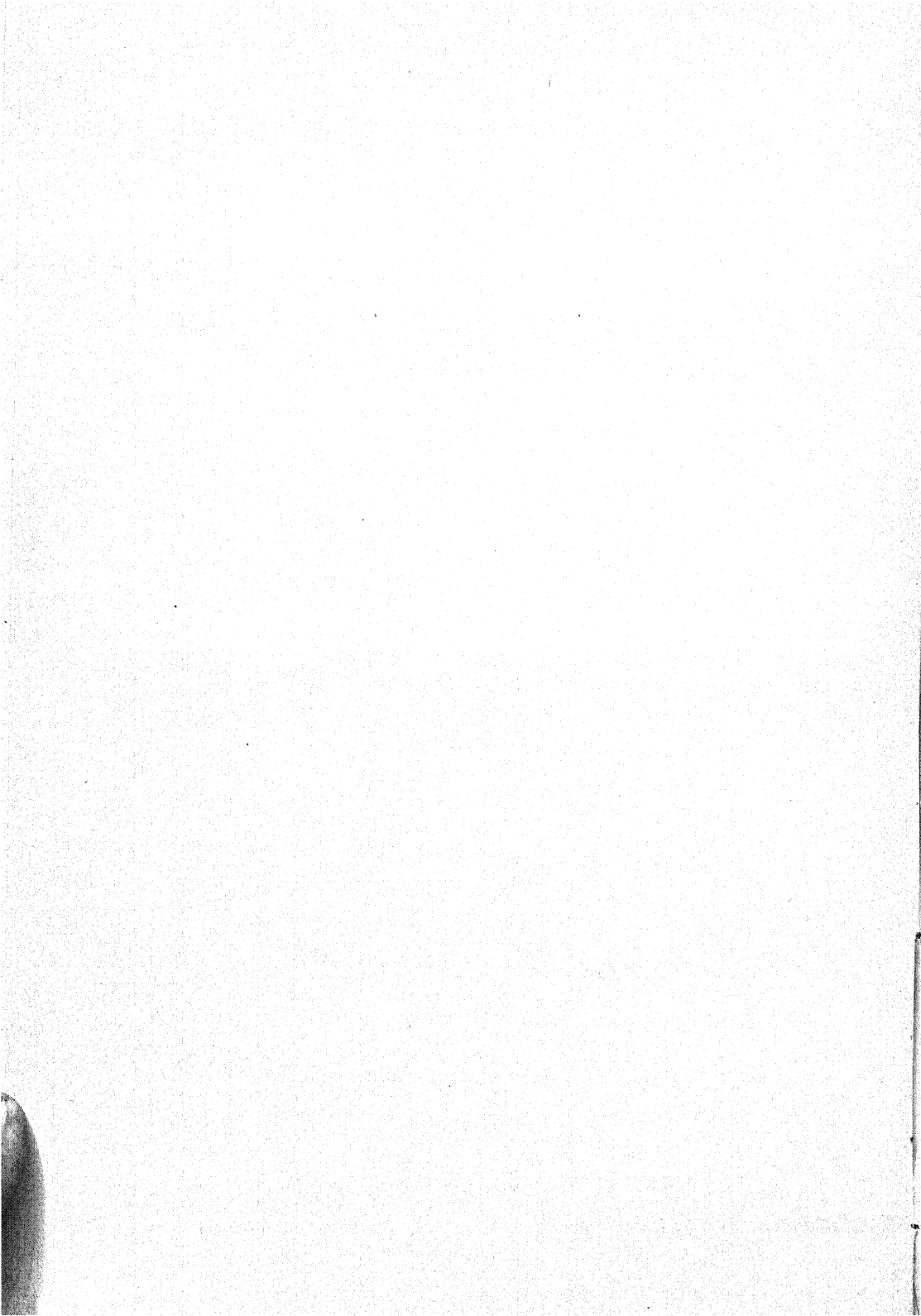
Materials for this paper were collected while I was working in the Botanical Laboratory of the University of Leeds. I have made extensive use of the works of Professors E. W. Sinnott, J. H. Priestley and I. W. Bailey. My thanks are due to them and also to the various other investigators whose works I have consulted and freely quoted.

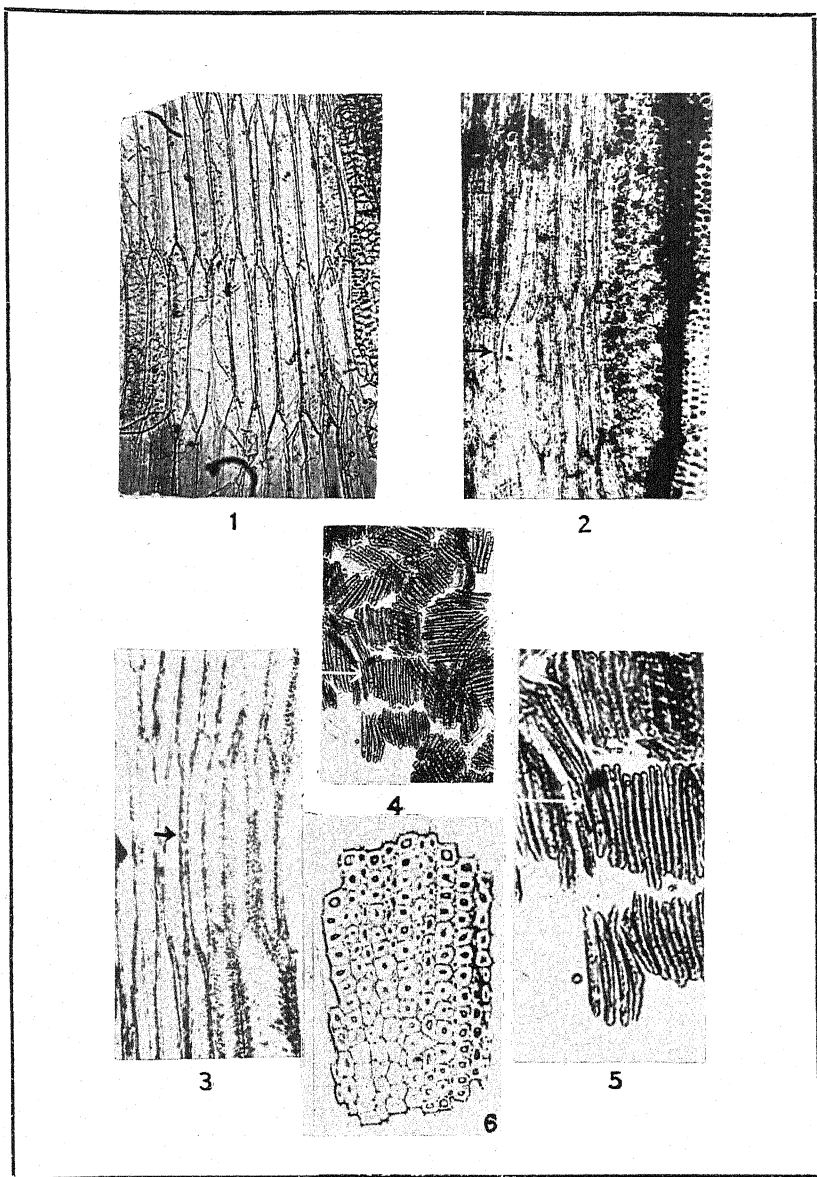
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EXPLANATION OF PLATE FIGS.

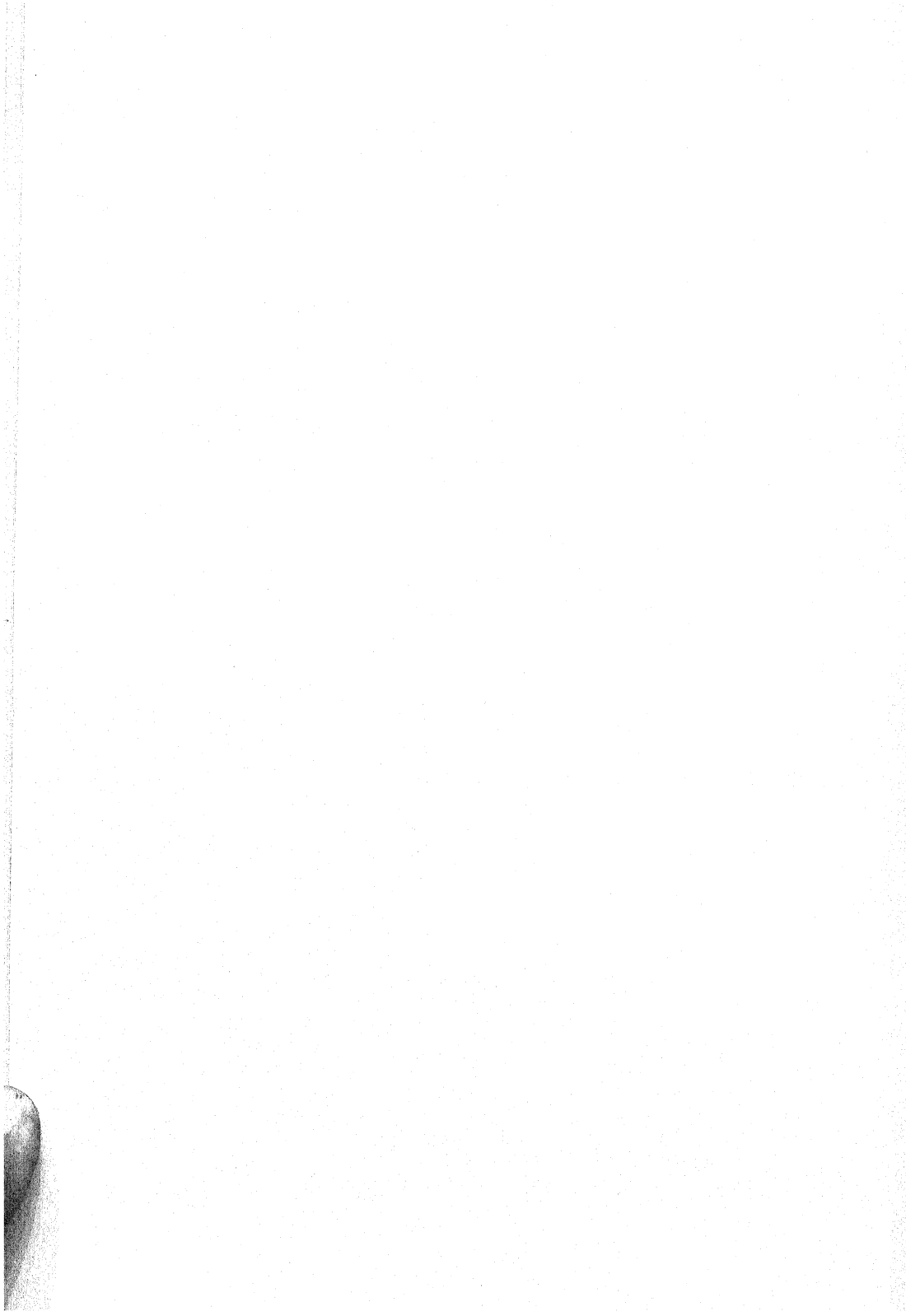
- Figs. 1, 2, 3. *Laburnum* sp. Intrusive growth during differentiation of a fibre from stratified cambial initials. Photomicrographs of stages shown in Text-figs. 5 A, B, C.
 Figs. 4, 5. *Heracleum Sphondylium*. Macerated cambium showing intrusive growth of the cambium initials; compare Text-fig. 6.
 Fig. 6. *Heracleum Sphondylium*. Photomicrograph of a patch of secondary xylem fibres in t.s.; intrusively grown fibre ends are seen in the right of the figure occupying the intercellular space regions.





G. P. MAJUMDAR

*THE INTRUSIVE GROWTH OF THE CAMBIUM CELLS AND THEIR
DERIVATIVES IN HIGHER VASCULAR PLANTS.*



GASTEROMYCETES OF THE WESTERN HIMALAYAS—I

BY SULTAN AHMAD, M.Sc.

Biological Laboratory, Government College, Rohtak

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IN 1935 the writer was privileged to accompany a botanical tour to Spiti organized by the Roerich Research Institute, Naggar, Kulu. The principle object of the tour was for the purpose of collecting seeds of grasses and of leguminous forage plants growing in that dry inner valley of the Himalayas. Incidentally a large number of flowering plants, ferns and fungi were also collected. The fungus specimens were generously handed over to the writer by the Director of the Institute.

The parasitic fungi were sent to Herr H. Sydow who has recently published the results (1938). The Gasteromycetes in that collection and a few others collected by the writer at Dalhousie, Chamba and Mussoorie and by Dr. R. R. Stewart from Kashmir have been identified and are reported in these papers.

FAMILY : HYMENOGASTERACEÆ

Melanogaster Corda.—This genus resembles the genus *Scleroderma* in the *Lakunar* type of development and was at one time placed by Fischer (1900, p. 334) in the family Sclerodermaceæ. But it differs markedly in its habit, gleba and spores and has therefore been transferred by Fischer (1933) to the family Melanogasteraceæ. The affinities of the genus, however, are so close to *Rhizopogon*, from which it differs only in the coloured spores and the arrangement of the gleba, that it has recently been included by Cunningham (1934) in the family Hymenogasteraceæ which he has amended to include the families Hysterangiaceæ and Hydangiaceæ.

1. *Melanogaster ambiguus* (Vitt.) Tulasne, *Fungi Hypogei*, 94, 1851.

Specimens globose or sub-globose, of nearly black colour, becoming much wrinkled on drying due to the deliquescence of the gleba. In young specimens cavities begin to appear in the centre where the deliquescence begins. The surface bears a number of dark brown fibrils which fall off in dried specimens. The wall is 160–230 μ thick and consists of gelatinised hyphæ and is soft to cut in dried plants.

Gleba jet black with a number of white tramal plates traversing it, more prominent near the periphery in the region where the gleba has not yet deliquesced. Spores broad, elliptic, more or less

pointed at the apex, dark brown, $10.5 \times 16.5 \mu$, with a very small hyaline cup-like pedicel at the proximal end. Basidia clavate, $7.5 \times 18.0 \mu$, 2-4 spored, spores borne on the short sterigmata, sterigmata $4-5 \mu$ in length.

Dalhousie—Chamba Road. Growing in groups a few inches below the ground. October 20, 1939. Leg. S. Ahmad.

The only other species of the genus recorded from India is *Melanogaster durissimus* Cke. which according to Lloyd (1921, p. 1064) is the same as *M. variegatus* (teste Mattiolo). The writer had recently a chance to examine the material of the species from Chakrata in the Herb. Crypt. Ind. Orient. of the Imperial Agricultural Research Institute, New Delhi.

The specimens are globose, very hard, showing no trace of deliquescence, with spores $3.75-4.5 \times 5.52-8.25 \mu$, generally rounded at the apex. The smaller size of the spores in this species is a good character to distinguish it from *M. ambiguus*.

2. *Rhizopogon rubescens* Tulasne, *Giorn. Bot. Ital.*,
ii, 58, 1844.

Specimens sub-globose to kidney-shaped, white in the fresh condition but turning to slightly pink on drying, without fibrils. Peridium wall $95-158.5 \mu$ in thickness, single layered, the outer part yellow and the inner pink in colour, the colour not changing in 7% KOH.

Gleba at maturity brittle, drying to pinkish or some shade of brown, the colour disappearing in old specimens, which then appears yellowish. Gleba cavities labyrinthiform, 12 to a millimetre, septa upto 90μ thick. Spores smooth, elliptic with two conspicuous oil drops, $2.25-3.0 \times 6.0-10.5 \mu$.

Dalhousie—Epigeous. Not common. October 20, 1939.
Leg. S. Ahmad.

The description of the specimen agrees in part with the description of *R. roseolus* presented by Coker and Couch (1928). According to these authors *R. roseolus* is closest to *R. rubescens* but can be distinguished by the following characters. In *R. rubescens* the colour of the peridium is citron yellow throughout while in *R. roseolus* yellow colour is limited only to the outer part, and the inner is distinct rosy-salmon. The two differ also in colour-changes in the gleba; in *R. roseolus* there is no change in the gleba upon sectioning, while in *R. rubescens* the surface changes to a distinct rosy-pink. The cavities of the gleba in *R. roseolus* are larger than in *R. rubescens*, and the septa are also considerably thicker, more gelatinized and much less sessile.

Lloyd (1923, p. 1170), on the other hand, remarks, "I am unable to clearly distinguish *R. roseolus* in sense of Dodge. It is a small form of *R. rubescens* with dark peridium."

Cunningham (1934) also fails to distinguish *R. roseolus* from *R. rubescens*. According to him the plant described by Coker and Couch is close to *R. rubescens*, if not identical with it, but he fails to establish the identity of *R. roseolus* in the original sense of Hollos. He states that the examination of three collections (listed in the text by Hollos) and identified by Dodge as *R. roseolus* shows that two belong to *R. rubescens* and one to *R. luteolus*, which illustrates the fact that *R. roseolus* has no character by which it may be recognised.

Rhizopogon luteolus Fr. is distinguished from *R. rubescens* by the gelatinized, hard, indurated and almost black gleba and the smaller glebal cavities filled with spores.

FAMILY : TYLOSTOMACEÆ

3. *Tylostoma mussooriense* P. Henn., *Hedwigia*,
XL, 337, 1901.

Peridium globose or sub-globose, 1.2 cm. in diameter and 1.0 cm. in height, reddish-brown. Exoperidium with numerous small granular warts, which fall off and give the endoperidium a beautiful verrucose appearance. Endoperidium very light-coloured opening by a well protruding tubular mouth. A small area round the mouth is perfectly smooth and if judged by this the endoperidium is likely to become smooth with age.

Stipe upto 6.5 cm. long, 0.25 cm. thick; strongly scaly, of brown colour, attenuated towards the apex, with a well-developed mycelial base.

Gleba pale-ferruginous; capillitium hyaline, rarely septate, not swollen at the nodes; spores globose or sub-globose, 3.8-4.95 μ in diameter, verrucose.

Mussoorie—Garhwal Road. On the ground in groups,
July 20, 1940. Leg. S. Ahmad.

The species was described by Hennings from specimens collected by William Gollan from Mussoorie, Arnigardh (5,500 ft.) on August 9, 1900. According to Hennings (1901) the species has an outward resemblance with *T. exasperatum* Mont., but is quite different as to mouth, etc. This is a very far fetched resemblance as in *T. exasperatum* in addition to the fibrillose mouth the peridium is furnished with large conical warts of the nature of spines, and in this last character it is unique. Its nearest relative, however, is *T. verrucosum* Morg., which has the same verrucose peridium, protruding tubular mouth, scaly stipe and verrucose spores. It is indeed difficult to separate the one from the other excepting by the size of the plants, a minor feature.

According to Lloyd (1906) it is close to *T. bonianum* Pat., but has much more slender stem and much roughened spores. It is not improbable that the three species, *T. verrucosum*, *T. mussooriense* and *T. bonianum* are local variants of the same species.

FAMILY: GEASTERACEÆ

4. *Geaster simulans* Lloyd, *Myc. Writ.*, 1, Aus. 17, 1905.

Exoperidium split to about the middle into 6-9 strongly hygroscopic rays, the exterior covered with debris or quite smooth. Endoperidium sessile, globose upto 1.3 cm. in diameter, glabrous, brown or almost dark-coloured; opening by an indefinite plane mouth.

Gleba ferruginous; columella indistinct; capillitium threads orange citrine, $3.9-4.6 \mu$ in diameter; spores, globose $3.9-4.6 \mu$ in diameter, apiculate, epispore fuscous, finely warty.

Rotang Pass (16,000 ft.). Solitary on the ground. *Leg. S. Ahmad.*

Characterised by the plane naked mouth and a strongly hygroscopic exoperidium. The species is distinguished from *G. fliformis* which it otherwise closely resembles in having smaller spores.

5. *Geaster Clelandii* Lloyd, *Myc. Writ.*, 5, 794, 1918.

Exoperidium split to about the middle into 6-8 acute and hygroscopic rays which fold over or under the endoperidium on drying. The exterior is covered over by vegetable debris. Endoperidium shortly pedicellate, depressed globose, upto 0.8 cm. in diameter, closely and minutely a separate, opening by a conical strongly sulcate mouth, seated on a distinct concolourous zone.

Gleba ferruginous; columella indistinct; spores globose or subglobose, $4.8-5.5 \mu$ in diameter, epispore pallid-brown, finely warty.

Jalori Pass (10,000 ft.).—Solitary on the ground. *Leg. S. Ahmad.*

Characterised by the sulcate mouth, hygroscopic exoperidium, and a pedicellate asperate endoperidium. The species resembles *G. Drummondii* and *G. Smithii* in general form but can be easily separated from the former by its pedicellate endoperidium and from the latter by its asperate endoperidium.

6. *Geaster mammosus* Fr., *Chev. Fl. Paris*, 359, 1836.

Exoperidium split to about the middle into 7-9 hygroscopic rays; revolute when the plant is wet and encloses the base of the endoperidium, the exterior is perfectly smooth. Endoperidium sessile, globose, upto 1.2 cm. in diameter, dark-brown, smooth, opening by a definite conical even fibrillose mouth seated on a definite concolourous or white area.

Gleba fuscous; columella indistinct; capillitium threads Sudan Brown (Ridgway), $3.4-4.8 \mu$ in diameter; spores globose, $3.35-4.46 \mu$ in diameter, epispore Sudan Brown (Ridgway), minutely verrucose.

Jalori Pass (10,000 ft.).—Solitary on the ground. *Leg. S. Ahmad.*

Characterised by a fibrillose mouth and a hygroscopic exoperidium. The species is distinguished from *G. arenarius* by its sessile endoperidium.

7. *Geaster triplex* Jungh. *Tidskr. Natur. Geschied.*, 7,

287, 1840; Syn. *G. lageniformis* Vitt.; *G. englerianus* P. Henn.

Exoperidium split to about the middle into 4-6 narrowly acuminate non-hygroscopic rays, which may be saccate or revolute. Fleshy layer brown, rimose, broken into transverse striæ, and at times forming a collar at the base of the endoperidium. Endoperidium sessile, 3 cm. in diameter, sub-globose, bay-brown, glabrous, finely pitted, membranous; opening by a fibrillose, mammose mouth seated on a broad, depressed, silky zone, marked by an upraised margin.

Gleba ferruginous; columella distinct clavate; spores globose, $3.36-4.83 \mu$ in diameter, epispore Sudan Brown (Ridgway), finely verrucose.

Khanag, Kulu Hills; Chamba.—Solitary on the ground.

Leg. S. Ahmad.

Characterised by the definite fibrillose mouth, non-hygroscopic cracked exoperidium, sessile endoperidium and the size of the spore. The unexpanded plants are ovate and with a long beak, and so can be easily distinguished from the closely allied species *G. saccatus* in which the unopened plants are globose, and without a beak.

As stated by Lloyd (1907, p. 310) and also by Butler and Bisby (1931) *G. englerianus* P. Henn., is a black form of *G. saccatus*. But according to Cunningham (1926) and Kambly and Lee (1936), it is not a form of *saccatus* but of *G. triplex* Jungh.

The following species were examined in the Herbarium of the Forest Research Institute, Dehra Dun:

Geaster saccatus Fr.

Kaghan Valley, Hazara. (No. 1886.)

This is a mis-determination, as the examination of the unexpanded plants shows a long beak characteristic of *G. triplex* (see under that species).

Geaster lilacinus Mass. *Bull. Miscell. Inform. Roy. Gard., Kew*, p. 166, 1899.

Dehra Dun (Gamble). (No. 105.)

The specimen was collected by Gamble from Dehra Dun in February 1898 and sent to Massee for determination, who described it as a new species. The main character on which the species is based and by which it differs (according to the author) from the closely allied species *G. hygrometricus* is the size of the spores. As remarked by Massee (1899) it (*G. lilacinus*) is "readily distinguished from this (*G. hygrometricus*) and every other known species by the very large size of the spores ($10-12 \mu$)".

The specimen in the Herb. Forest Res. Institute was recognised at a glance as the common *Astræus hygrometricus*. The examination of the spores confirmed this first impression; they are globose, verrucose, dark-brown, $5.54-13.6 \mu$ in diam., the mean being 10μ . This is the usual size of the spores in specimens from Dalhousie, Kulu and Mussoorie. This is further confirmed by the description of *A. hygrometricus* presented by other writers. Lloyd (1902) records the spores as $8-12 \mu$, while Kambly and Lee (1936) state that the spores vary extremely in size, from $4-5 \mu$, more commonly from $7-12 \mu$, averaging about 9μ .

There appears to be no justification whatsoever for keeping *Geaster lilacinus* Mass. as a species distinct from *Astræus hygrometricus* and it has therefore been reduced to synonymy (see below).

FAMILY : ASTRÆACEÆ

8. *Astræus hygrometricus* (Pers.) Morgan, *Jour. Cinn. Soc. Nat. Hist.*, 12, 19, 1889-90; Syn. *Geaster hygrometricus* Pers.; *Geaster lilacinus* Mass.; *Astræus stellatus* Scop.

Exoperidium split to about the middle into 6-12 strongly hygroscopic rays, perfectly smooth on the exterior; fleshy layer breaking into irregular areas by deep grooves; falling away completely in some specimens. Endoperidium sessile, depressed globose, upto 2.5 cm. in diameter, with surface felted and often marked by pitted areas; dirty-white or gray; opening by an irregularly torn aperture.

Gleba dark-brown; columella absent; capillitium threads hyaline long branched, $3.8-6.65 \mu$ in diameter; Spores globose, $6.5-12.5 \mu$ in diameter, dark brown, verrucose.

Dalhousie—Chamba Road; Khanag, Kulu Hills; Mussoorie.—solitary on the ground. Very common. Leg. S. Ahmad.

The unopened specimens are very hard and globose and as stated by Lloyd (1902) "are liable to be taken at first for a species of hypogaeal fungi, or on examination under a microscope, for an undeveloped *Scleroderma*".

The species has been variously referred to by different workers. Lloyd (1902) put it in the Geastræ, but Fischer (1933) referred it to the family Calostomaceæ with the genus *Calostoma*. As its affinities with other Gasteromycetes are not clear, Martin (Kambly and Lee, 1936) has recently proposed a separate family *Astræaceæ* for it.

FAMILY : LYCOPERDACEÆ

9. *Mycenastrum corium* (Guers.) Desvaux, *Ann. Sci. nat.*, 2, XVII, 143, 1842; Syn. *M. spinulosum* Peck.

Plants globose, sub-globose or pyriform, upto 12 cm. in diameter. Exoperidium formed of a white thin fugacious layer; endoperidium corky, 2-4 mm. thick, smooth, polished, bay-brown, opening by an irregular break at the apex at maturity, but more

often breaking away from the point of attachment and rolling on the ground like a "Bovista". Sterile base none.

Gleba dark-brown, pulverulent; capillitium of short separate threads abundant, unseptate, branched, beset with numerous spiny processes; spores globose, $7.5-10.5 \mu$ in diameter, epispore chestnut-brown, densely echinulate.

Ani (3,000 ft.), Kulu Hills; Chamba—Dalhousie Road.—Solitary or in groups on the ground. Common. Leg. S. Ahmad.

A cosmopolitan species characterised by its large globose form, a "Bovista"-like habit, thick and corky endoperidium and spiny capillitium.

10. *Bovista plumbea* Pers. *Syn. Fung.*, 137, 1801.

Peridium globose or sub-globose, 3.1 cm. in diameter and 2.6 cm. in height. The surface in the young specimen breaks up into small granules as it dries; finally, the exoperidium peels off in large pieces; endoperidium smooth, firm, lead-coloured. The mouth is not yet developed in the specimens.

Gleba olivaceous; capillitium threads separate, dichotomously branched, with slender tapering branches; spores sub-globose, smooth, $4.75-5.9 \mu$, pedicellate, pedicels $8.6-10.85 \mu$ in length.

Sonamarg, Kashmir, 9,000 ft. Leg. R. R. Stewart.

The species is easily recognised by its lead-coloured endoperidium, and oval spores with long pedicels.

A specimen has already been recorded under this name from Mussoorie, India (cfr. Butler and Bisby, 1931). The record is based on a specimen collected by Gollan and sent to Dr. Hennings for determination, who referred it to this species. One half of the same specimen was given to Lloyd by Dr. Hennings who refers it to *Bovistella* and calls it *Bovistella Henningsii*. He remarks "I feel sure it should not be referred to *Bovista plumbea*, as it has a cortex different from all known Bovistas (minute fasciculate persistent spines)....etc." The plant recorded by Butler and Bisby (1931; should be therefore designated *Bovistella Henningsii* Lloyd. The first record of *Bovista plumbea* Pers. is therefore the one now collected by Dr. R. R. Stewart from Kashmir.

11. *Bovistella lycoperdoides* (Oke.) Lloyd, *Myc. Writ.*, 2, 280, pl. 87 (f. 5, 6), Aug. 1906 (as *B. lycoperdoides*); *Mycenastrum lycoperdoides* Oke. *Grev.* 13, 6, 1884; *Scleroderma Cookei* de Toni in *Sacc. Syll. Fungorum*, 7, 140, 188.

Peridium sub-globose or pyriform, with a well-developed stem-like sterile base. Exoperidium white, persisting in the ripe specimens as beautiful white specks; endoperidium light-brown, opening by a well-developed mouth.

Gleba-olivaceous-brown; capillitium olivaceous, of dichotomously branched separate threads with tapering branches; spores globose, $4.6-5.5\ \mu$, smooth, pedicellate, pedicles $8.5-12.6\ \mu$ in length.

Sonamarg, Kashmir, 9,000 ft. Amongst moss. *Leg. R. R. Stewart.*

The specimen has been variously referred to in the past by different writers. It was originally collected from Nila Valley, Garhwal, Himalayas (12,000 ft.) and described by Cooke as *Mycenastrum lycoperdoides*. It was later compiled by de Toni in Saccardo as *Scleroderma Cookei*, with which it has not the remotest connection and is so recorded by Butler and Bisby (1931). It was not until 1906 that Lloyd examined the specimens at Kew and assigned it to its natural position.

12. *Bovistella bovistoides* (Cke. and Mass.) Lloyd, *Myc. Notes*, 247, 1906.

Peridium globose, sub-globose or pyriform, upto 3.2 cm. in diameter and upto 4 cm. in height, with a distinct rooting base. Exoperidium breaking up into small granules which fall off completely leaving the endoperidium smooth, of brownish colour. Endoperidium papery-membranous, light or dark-brown, paler at the base, opening by an irregular torn aperture, $0.4-0.8\text{ cm.}$ in diameter.

Gleba-dark brown; capillitium of free dichotomously branched threads with tapering branches, reddish-brown; spores sub-globose, smooth, $4.2-5.6\ \mu$; pedicellate, pedicel nearly hyaline, curved, upto $13.5\ \mu$ in length.

Kulu; Dalhousie—Chamba Road.—Amongst moss. Common. *Leg. S. Ahmad.*

13. *Calvatia gigantea* (Pers.) Lloyd, *Myc. Notes*, 1. *Lyc. Aust.*, 36, 1905; *Lycoperdon giganteum* Pers., *Syn. Fung.*, 140, 1801.

Plants sub-globose to pyriform, upto 6.3 cm. in diameter. Exoperidium minutely furfuraceous, yellowish. Endoperidium light brown opening by a small torn aperture. Sterile base poorly developed and absent in some specimens.

Gleba yellowish; capillitium threads long, sparingly branched, septate, olivaceous; spores globose, $3.9-5.2\ \mu$ in diameter, finely verrucose.

Sonamarg, Kashmir (9,000 ft.). *Leg. R. R. Stewart.*

Characterised by the absence of a diaphragm, by scanty sterile base and yellowish gleba. The specimens differ from the typical forms in the peridium opening by a definite mouth and in not flaking away in small pieces. That is perhaps why it has often been reported as *Lycoperdon giganteum*. The specimens also do not compare in size with European and American specimens which are often as big as being three feet in diameter.

14. *Calvatia cœlata* (Bull.) Morgan, *Jour. Cin. Soc. Nat.*

Hist., 12, 169, 1889.

Peridium depressed-globose, 12 cm. in diameter and 4.2 cm. in height. Exoperidium areolated into thick conical warts; endoperidium thin, smooth, pale below and dark-brown above, opening by a well-developed lacerate mouth, of darker colour than the rest of the peridium. Sterile base well developed, becoming split from the base upwards into small recurved lobes in the specimen at hand, thus assuming the form of a huge *Geaster*.

Gleba not at all pulverulent, but forming a light cohesive spongy mass, bay-brown; capillitium threads branched, occasionally with a wavy margin, Sudan Brown (Ridgway), 3.6–5.58 μ in diameter; spores globose or sub-globose, 4.65–5.58 μ in diameter, epispore Sudan Brown (Ridgway), smooth.

Babeh Pass (18,000 ft.); Bashahr State.—On the ground.

Leg. S. Ahmad.

The specimen shows some differences from the description of other authors. The gleba is not pulverulent, whereas Lloyd states (1905, p. 36) that "the gleba of this species has very little cohesiveness and falls out from the specimen so readily that they are usually the dirtiest puff-balls we receive". The capillitium threads are of almost the same diameter as the spores, while according to Kambly and Lee (1936) and Lloyd (1905, p. 36) they are "two or three times as thick as the spores." In this character it resembles *C. Cranii-formis*, but it differs from it in the nature of the exoperidium and the size of the spores.

ACKNOWLEDGEMENTS

The writer would like to express his appreciation of the kind interest taken by Dr. R. R. Stewart, Principal, Gordon College, Rawalpindi; Dr. N. L. Bor, Forest Botanist, Dehra Dun, and Dr. B. B. Mundkur of the Imperial Agricultural Research Institute, New Delhi.

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IMPORTANCE OF GROWTH-PROMOTING SUBSTANCES IN THE METABOLISM OF *PYTHIUM INDIGOFEARÆ* BUTLER

BY R. K. SAKSENA

(Botany Department, University of Allahabad)

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INTRODUCTION

It was Pasteur¹² who introduced the use of synthetic nutrient media and since then a large amount of work has been done with a view to determine the elements essential for the growth and reproduction of fungi. In the preparation of synthetic media usually a nitrogen source, a carbon source and certain essential minerals are provided. It is now well known that many fungi are unable to grow in such synthetic media unless small quantities of certain other substances, known as accessory growth substances, are added. Thus fungi may be either autotrophic or heterotrophic with respect to accessory substances, the former are able to synthesize the accessory substances from the simple ingredients of the synthetic media, while the latter must be supplied with them from the outside.

A large amount of data has been accumulated by various workers indicating the necessity of an extraneous supply of growth-promoting substances for many fungi. Early work on the subject has been well summarized by Bonner,¹ Lilly¹¹ and Steinberg.²¹ The recent contributions on the subject are those of Hawker,^{5,6} Leonian and Lilly^{9,10} and of Robbins.¹³⁻¹⁶

In a previous paper¹⁸ the writer communicated that *Pythium indigoferæ* did not grow in a medium containing K_2HPO_4 , $MgCl_2$, $6 H_2O$, K_2SO_4 , NH_4NO_3 , pure dextrose and distilled water and wrote, "It is remarkable that *Pythium indigoferæ* grows only where peptone is available as source of nitrogen. It is possible that the fungus does not grow.....on account of the lack of an accessory factor in the medium. It is also likely that it requires a fairly complex nitrogenous substance like peptone as source of nitrogen." The present investigation is an attempt to extend our knowledge concerning these problems.

EXPERIMENTAL METHODS

The methods used were in general those of the early papers.^{18,19} Experiments were run in triplicate and each experiment was repeated at least once. As far as possible guaranteed reagents were used. Thiamin (Vitamin B_1) was obtained from Merck & Co. Since yeast extract could not be obtained Fleischmann's pure dry yeast (from

A. H. Thomas Co., Philadelphia, U.S.A.) was used. It was well ground before it was added to the media. The basal medium, which will afterwards be referred to as medium A, consisted of 0.5 gm. each of K_2HPO_4 , $MgCl_2 \cdot 6 H_2O$ and K_2SO_4 , 5.0 gm. of pure dextrose (dextrosol of Corn Products Co.) and 1000 c.c. of distilled water. It has already been demonstrated^{2, 17, 19} that dextrosol is free from the significant amount of thiamin, pyrimidine and thiazole. Yeast and extracts of casein and lentil were added to media before autoclaving while thiamin was added after autoclaving. The range of temperature during the experiments was 25°–30° C. The medium for the source of inoculum was agar (from Messrs. Townson and Mercer, Ltd., London). The hydrogen-ion concentrations of the acidic nutrient solutions were adjusted to pH 7 before autoclaving. For the solidification of media Difco bacto-agar was used.

The methods used by Farries and Bell⁴ for hydrolysing peptone and casein, preparing the synthetic protein and for extracting the active substances from butter-milk were adopted.

A mixture of amino-acids was prepared after the method of Leonian and Lilly.⁸ It consisted of arginine, glutamic acid, aspartic acid in 2 parts each, alanine and glycine in one part each.

The writer is thankful to his colleague, Dr. R. N. Tandon, who kindly gave lentil extract prepared according to the process used by Buston and Pramanik.³

Growth in hydrolysed peptone.—Difco bacto-peptone (10 gms.) was gently warmed for one hour with four times its weight of 22.5 per cent. pure sulphuric acid, after which it was heated under a reflux condenser for 24 hours. The liquid, while still hot, was neutralized in order to prevent the formation of piperazines. The liquid was diluted to five times its volume and a hot saturated solution of baryta was added. To recover the greater part of nitrogen the precipitate was washed by boiling with distilled water. The combined filtrates and washings were made faintly alkaline and finally the last traces of baryta were precipitated with dilute sulphuric acid. The total volume of the liquid thus obtained came to 435 c.c. of pH 5.1.

The first problem was to determine whether the fungus required a fairly complex nitrogenous molecule such as peptone, or whether it would grow in a mixture of its simple hydrolytic products. The results of experiments showed that the organism grew well in hydrolysed peptone and also in the medium A, to which the hydrolytic products were added. These results clearly indicate that it does not require a complex nitrogenous molecule like peptone.

Growth on a synthetic protein.—The next problem was to find out whether the fungus would grow on a synthetic protein (a mixture of amino-acids) with constituents approximately equivalent to those of Lactalbumin, according to the analyses of Jones and Johns.⁷

The synthetic protein consisted of:—

Mono-amino-acids	.. {	Glycine	0.25%
		Alanine	0.25%
		Leucine	0.25%
Dicarboxylic acids	.. {	Glutamic acid	0.18%
		Asparatic acid	0.18%
Cyclic compounds	.. {	Proline	0.13%
		Phenylalanine	0.05%
		Tryptophane	0.03%
		Tyrosine	0.05%
Protein bases	0.67%

The following media were prepared:—

B. 0.5 gm. of synthetic protein + 50 c.c. of distilled water.

C. Medium B + Difco bacto-agar 2%.

D. Medium A + synthetic protein 0.2 %.

None of the media so prepared gave any growth of the fungus. The results show that the synthetic protein lacks in some accessory growth substances, which are essential for the growth of the organism and which are present in the hydrolytic products of the Difco-bacto-peptone.

On account of the difficulty and expense of obtaining sufficient quantities of the various costly amino-acids the synthetic protein was used in low concentration and the number of repeat experiments had also to be reduced.

Effect of thiamin on growth.—To medium A was added 0.2 per cent. of NH_4NO_3 and 10 c.c. of this, in each case, were mixed with a sterile solution of thiamin (giving 2 international units per tube in one set of experiments and 10 units in another). None of these nutrient solutions was found favourable for the growth of the fungus.

The experiments were repeated with the addition of the mixture of amino-acids (0.1 gm. per 100 c.c. of the nutrient solutions) but the results obtained were the same. From these results it is clear that neither the addition of thiamin nor of thiamin and amino-acids induces growth.

Effect of casein extract on growth.—1500 c.c. of 95 per cent. alcohol were slowly added to 500 c.c. of butter-milk, which was stirred with a glass rod, and the whole was filtered. The precipitate was taken up in water and reprecipitated thrice, finally washed with alcohol and dried. This will be referred to as casein. It was hydrolysed in the usual way.

All the alcoholic filtrates were mixed together and were evaporated to dryness, treated with absolute alcohol to precipitate the last traces of casein and filtered. (The alcoholic filtrates contained a good amount of salts, which precipitated on evaporation. These

were discarded.) The filtrate was freed from alcohol and evaporated down to 250 c.c. This liquid will be referred to as casein extract. The following media were used :—

- E. Butter milk.
- F. 5.0 gm. casein + 100 c.c. distilled water.
- G. 100 c.c. medium A + 0.2 gm. casein.
- H. Hydrolysed casein.
- I. 100 c.c. medium A + 0.2 gm. NH_4NO_3 + 5 c.c. casein extract.
- J. 100 c.c. medium F + 5 c.c. casein extract.
- K. 100 c.c. medium G + 5 c.c. of casein extract.
- L. 100 c.c. medium H + 5 c.c. of casein extract.
- M. 0.5 gm. synthetic protein + 50 c.c. of distilled water + 2.5 c.c. casein extract.

In another set of experiments Difco bacto-agar 2% was added to these media.

The results of experiments indicated that *P. indigofera* was capable of growing well in butter-milk, and in those media in which the source of nitrogen was either NH_4NO_3 or the casein, or the hydrolysed casein, or the synthetic protein, *only when the casein extract was added to the media*, and that it preferred the casein to NH_4NO_3 as source of nitrogen. It did not give appreciable growth in media F, G and H. It is clear from the above results that (i) the casein extract contains some active growth-promoting substances in the absence of which the fungus can not grow, (ii) the organism does not require a complex nitrogenous substance such as casein, as is shown by the fact that it grows in the hydrolytic products of casein provided the casein extract is added as in medium L, and (iii) the synthetic protein lacks in the active substances for growth.

Effect of lentil extract and yeast on growth.—In a previous paper¹⁸ it was communicated that *P. indigofera* was incapable of growing on Difco bacto-agar but the results of the present experiments show that the addition of small quantities of the lentil extract or yeast to the bacto-agar, to medium F and also to medium A containing NH_4NO_3 as source of nitrogen, induce the growth of the fungus. In 0.005 gm. of the yeast per 100 c.c. of distilled water it did not grow but when this amount was added either to 100 c.c. of 2% bacto-agar or to 100 c.c. of medium A (with NH_4NO_3) it grew, though poorly. The minimum quantity of yeast required for a fair growth of the fungus was 0.01%.

It is now a well-established fact that the lentil extract contains accessory growth factors⁵ and that outstanding among the known compounds of the yeast are the members of the vitamin B complex and amino-acids.⁸

CONCLUSION

Pythium indigoferæ is one of those fungi which do not respond to thiamin only and which require an external supply of a complex of growth-promoting substances. Leonian and Lilly⁸ have demonstrated that *Coprinus lagopus*, *Nyctalis asterophora*, *Pilaira moreaui* and *Pleurotus corticatus* require a mixture of certain amino-acids besides thiamin, and that *Allomyces javanicus*, *Ascobolus viridulus*, *Ashbya gossypii*, *Ceratostomella multi-annulata*, *Dipodoascus uninucleatus*, *Ophiobolus oryzinus*, *Saprolegnia parasitica*, *Sordaria fimicola*, *Spormoia intermedii* and *Thraustotheca clavata* grow only when the yeast extract is added. According to Schopfer and Blumer²⁰ *Ustilago zeæ*, *U. tritici*, *U. levis*, *U. nuda*, *U. hordei*, *U. avenæ*, *U. bromivora* and *U. longissima* and according to Hawker⁶ *Melanospora destruens*, *Podospora corvula*, *Sordaria* sp. and *S. fimicola* are not benefited by thiamin.

SUMMARY

1. *Pythium indigoferæ* does not grow on a synthetic medium in which the source of nitrogen is not peptone.

2. It does not require a complex nitrogenous molecule as such, as shown by the fact that it grows on a medium in which peptone is completely hydrolysed to its constituent amino-acids.

3. While there is good growth on peptone and hydrolysed peptone there is no growth on a mixture of amino-acids which is representative, as far as possible, of the hydrolytic products of a complete protein.

4. The addition of thiamin to the synthetic medium, in which the source of nitrogen in amino-acids or ammonium nitrate, does not induce growth.

5. The fungus is capable of growing in butter-milk, but the utilization of casein of butter-milk is conditional on the presence of a growth-promoting substance, which is easily separable from the milk by alcoholic precipitation.

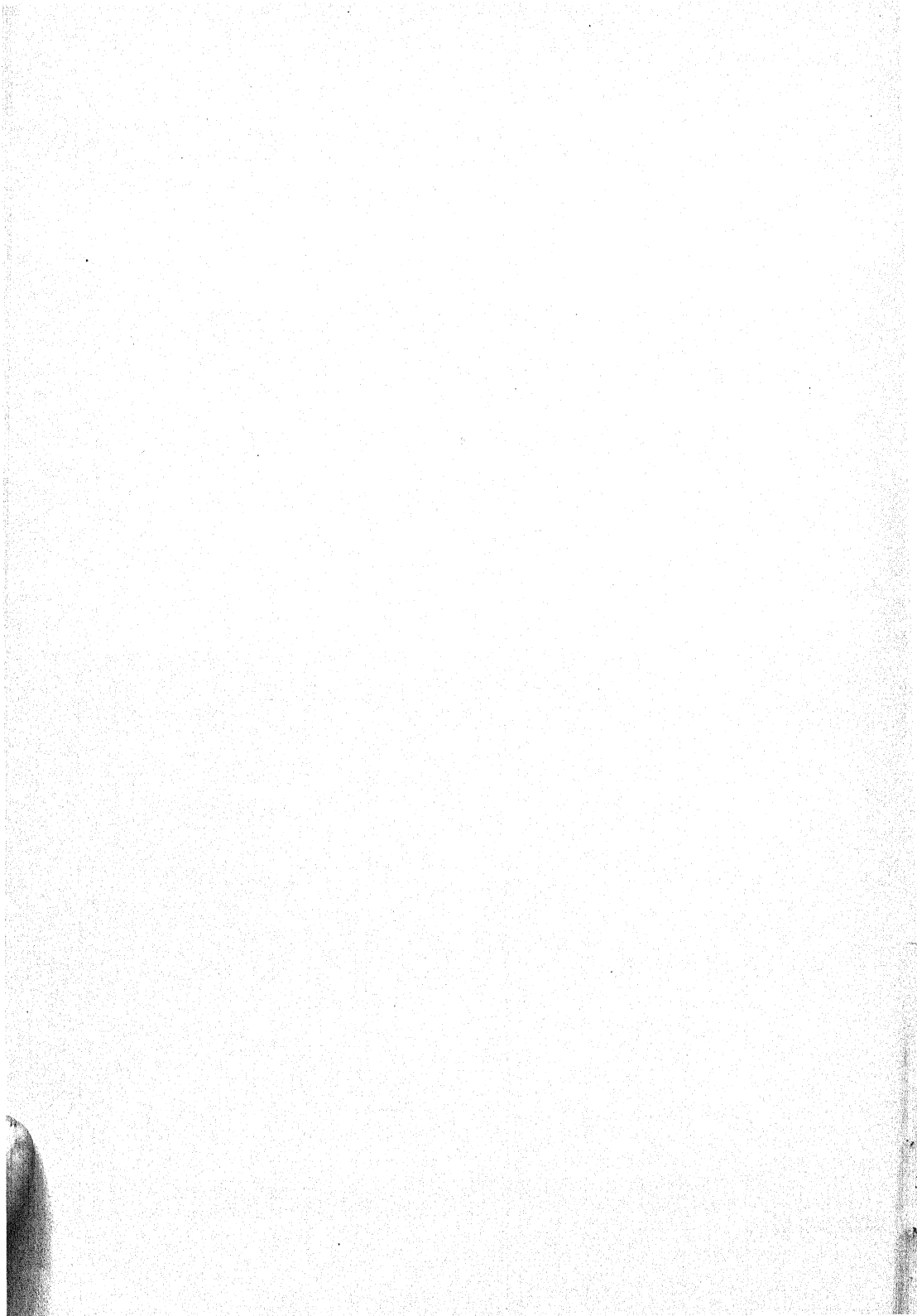
6. The organism can grow on a synthetic medium, in which the source of nitrogen is ammonium nitrate, when casein extract or lentil extract or yeast is added to the medium.

7. The results of the experiments clearly show that the organism does not respond to thiamin, but requires an extraneous supply of a complex of growth-promoting substances for its growth.

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A NEW GYMNOSPORIA FROM BASTAR
STATE, INDIA

Gymnosporia Bailadillana Narayanaswami et Mooney
spec. nov. (Celastraceæ—Celastrææ)

BY V. NARAYANASWAMI

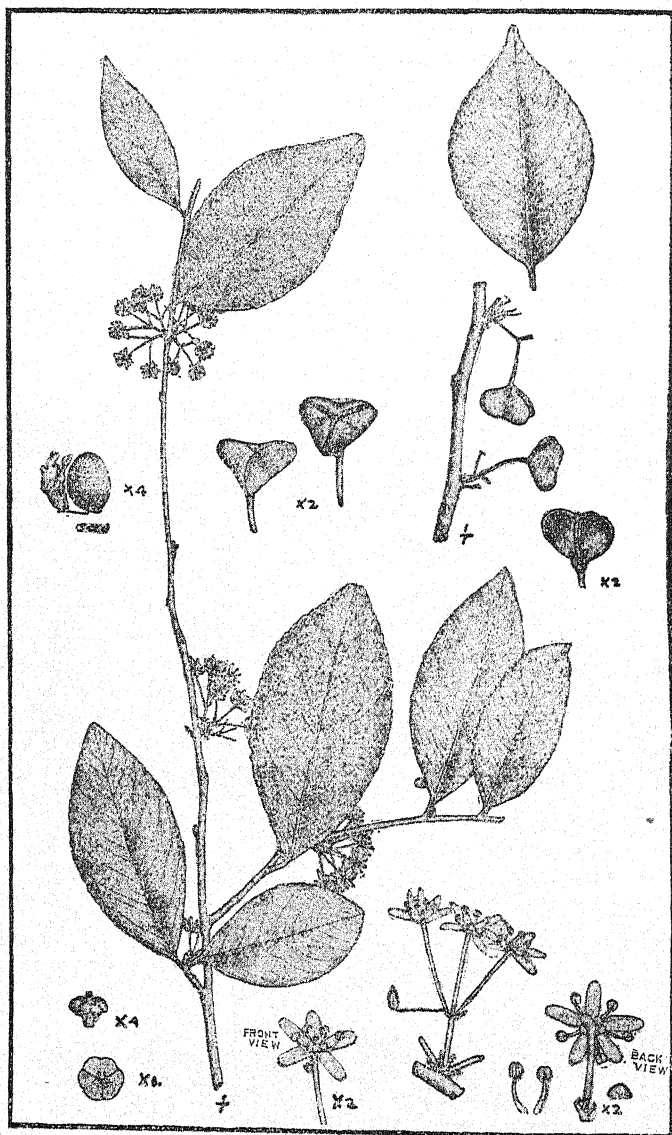
Botanical Survey of India

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GYMNOSPORIÆ Falconeri affinis, sed ab ea partibus omnibus glabris, foliis elliptico-ovatis, pedunculis pauci-florum cymorum brevissimis, fructibus majoribus distinguitur.

From a shrub to a small tree, up to 7 metres high ; branches with few short slender straight axillary thorns or sometimes almost thornless, thorn 3-13 mm. long ; branches dark violet to dark brown, striate, irregularly angled near the tips, sometimes with numerous white lenticles on some branches, quite glabrous or indistinctly canescent ; leaves simple, alternate, 5-9 cm. long by 2-5.2 cm. broad, shortly petiolate ; petiole 5-8 mm. long, jointed below, lilacy white, turning brown later ; leaves elliptic to elliptic ovate, obtuse or shortly obtusely acuminate, seldom acute, base cuneate, margin minutely glandular crenulate ; main nerves up to 15 ; midrib lilacy white, turning brown later ; finely reticulate on both sides, glaucous above, sometimes shining above and greenish brown, brown below. Inflorescence in short peduncled simple or dichotomous cymes of three or less small flowers ; peduncles axillary, from old leafless axils or at the base of short lateral leafy or leafless branchlets ; solitary but more often in fascicles of two-four ; bracteate and bracteolate ; bract and bracteole, minute less than a mm. long, reddish brown, ovate and minutely fimbriate on the margins ; peduncle up to 5 mm. long, lilacy white, turning brown ; pedicel clavate, jointed below, 5 mm. long ; bud 3 mm. long, oblong or globose ; sepals 5, imbricate, 1 mm. long, broadly ovate, round obtuse, minutely ciliate at the margin ; petals 5, 3 mm. long, imbricate, oblong, truncate below, round obtuse above, minutely crenulato-serrulate on the round tip, white with orange centre ; stamens 5, inserted below the orange coloured disk and alternate to the petals ; filament 2 mm. long, anther oblong or round, less than a mm. long, basifixed, disk thick, 5 crenate lobed ; ovary 3 celled, half or fully sunk in the fleshy disc ; 2 ovules in each cell ; style 3 lobed, lobes bifid ;

stigma simple minute; fruit 3 lobed obtriangular loculicidal capsule, 6-7 mm. long, up to 10 mm. broad, slightly depressed above in the centre, cuneate below, more or less transversely striate dorsally, reticulate at the margins; dehiscence loculicidal from above; seeds 2 in each cell, collateral ovoid, shining, arillate; aril completely covering young seed, splitting irregularly when mature, loosely hanging at the base of the seed.

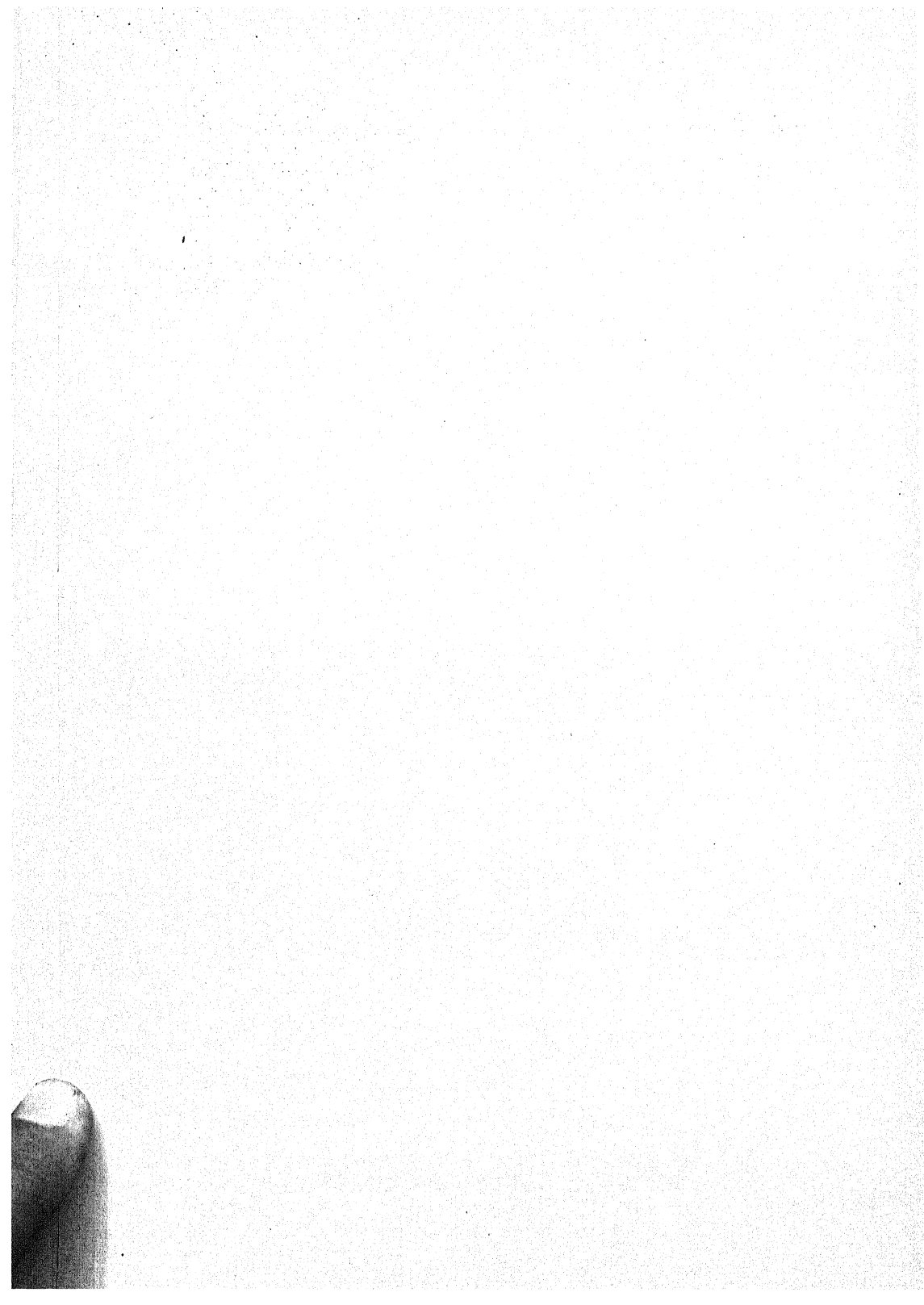


NEW GYMNOSPORIA FROM BASTAR STATE, INDIA 193

Type. H. Mooney No. 890 from Bailadilla Hill, Bastar State (Orissa) (in the Calcutta Herbarium).

Other collections are H. F. Mooney Nos. 390 and 900.

In the dark glens and shady ravines or in open moist valleys on the Bailadilla Hill of the Bastar State in Orissa, between 3000 and 3500 ft. elevations.



THE BIOLOGICAL SPECTRA OF THE MATHERAN AND MAHABALESHWAR FLORA

BY F. R. BHARUCHA

AND

MISS D. B. FERREIRA

Department of Botany, Royal Institute of Science, Bombay

Received.....

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Introduction

IN 1905, Christen Raunkiaer published the first comprehensive account of a Life-form System of plants which remains to-day in principle, the same. This system is simple and different from all previous life-form systems (except that of Krause) by being based mainly on *only one single feature*, namely, "the protection of the bud of the shoot-apices to the unfavourable season". Theoretically he defined the life-form as "the sum of the adaptation of the plant to the climate".²⁹

Raunkiaer worked out his life-form system with the definite purpose of using the flora of a given tract of a country as an exact indicator of its climate, for he firmly believed "that the plant-climate is characterized by the statistics of life-forms, that is to say, that the life-forms best adapted to a certain climate will form a higher percentage of the flora than others".²⁹

On the basis of extensive investigations of the life-form composition of various floras, he attempted to define the main plant-climates of the earth according to the percentages of the various life-forms. Thus he distinguished four main plant-climates :—

TABLE I

Regions	The percentage of distribution of the species among the life-forms											
	No. of Sp.	*S	E	MM	M	N	Ch	H	G	HH	Th	Plant-climates
St. Thomas & St. Jan	904	2	1	5	23	30	12	9	3	1	14	Phanerophyte
Death Valley, California	294	3	2	21	7	18	2	5	42	Therophyte
South Labrador	334	3	3	8	9	48	12	11	6	Hemicryptophyte
Jan Meyen	137	32	57	8	..	3	Chamaephyte
Normal Spectrum	1000	1	3	27	..	15	9	26	4	2	13	

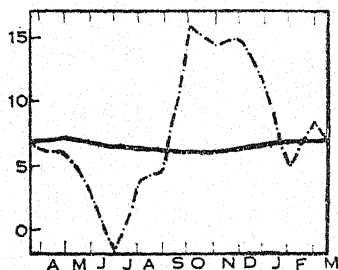
- (1) Phanerophyte-climate in the tropics.
- (2) Therophyte-climate in the winter rain regions of the tropics.
- (3) Hemicryptophyte-climate in the greater part of the cold-temperate zones. And
- (4) Chamæphyte-climate in the cold zones.

While the first three plant-climates were characterised by a larger percentage of phanerophytes, therophytes and hemicryptophytes respectively than any other life-form percentage in the respective floras, the chamæphyte-climate was characterized by a higher percentage of chamæphytes than its corresponding percentage in the Normal Spectrum. Raunkiaer called the statical conspectus of the life-forms of a flora, that is to say, the percentage distribution of the species among the life-forms of any flora, a "Biological Spectrum" or "Phyto-climatic Spectrum" and he further defined the *Normal Spectrum*²⁹ as "the percentage relations between the life-forms of all the phanerograms of the world" (*ibid.*, p. 429).

Thus Table I represents the biological spectra of four regions together with their corresponding Plant-climates.

The climatic features of each of the above four regions are as follows :—

(1) In the tropical region, the climate is constantly warm and constantly moist, that is to say, both the temperature and precipitation curves are high all the year round. This is seen in the Hydrotherm Figure for a tropical region. The hydrotherm figure for any region according to Raunkiaer is a figure showing the relationship between the temperature curve, plotted in degrees



*Fig. 1. Hydrotherm figure for the East Coast of Sumatra

* Figs. 1, 2 and 3 are based on Figs. 2, 5 and 1 respectively from "The Life-forms of Plants and Statistical Plant Geography," by C. Raunkiaer, Oxford University Press.

centigrade and the precipitation (rain) curve, plotted in centimetres, in the same graph. Unfortunately the hydrotherm figure for St. Thomas and St. Jan are not known. As such the hydrotherm figure for a climatically alike place like Sumatra indicates that the conditions there are favourable throughout the year, there being no unfavourable season. As Phanerophytes are the least protected among all plants, they are found in those portions of the earth which are most favoured climatically or using Raunkiaer's expression "Phanerophytes are predominantly plants of favourable climates". The hydrotherm figure for Sumatra should therefore correspond with that for St. Thomas and St. Jan.

(2) In the sub-tropical winter rain region, both the *temperature* and the *precipitation* curves show a conspicuous trough but the trough of the latter occurs at a different season from that of the former, thus causing a dry summer and a more or less humid winter. Therophytes which occur in this region are plants of the favourable season or "summer plants," as Raunkiaer puts it. They survive the unfavourable season in the form of seed and complete their life-cycle within a single favourable season. The hydrotherm figure for South Italy (Temperature curve is that of Naples) indicates these features of the Sub-tropics, and as such should correspond to that of the Death Valley, California, for which the Biological Spectrum is given in Table I.

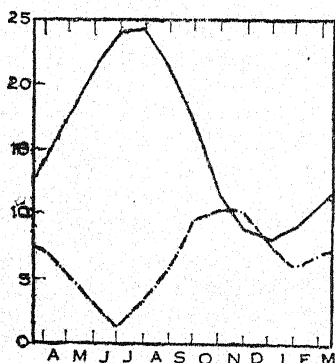


Fig. 2. Hydrotherm figure for South Italy

(3) In the cold-temperate regions, the *temperature* curve demarcates the region, as the precipitation is usually high in relation to the temperature. Hemicryptophytes which grow predominantly in this region are able to survive the unfavourable season as their buds are actually embedded in the ground. In this case the hydrotherm figure for Denmark is given.

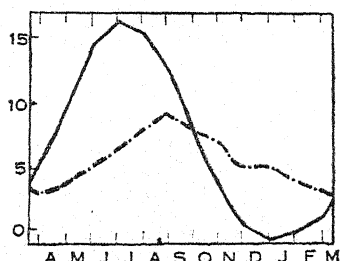


Fig. 3. Hydrotherm figure for Denmark

(4) In the fourth region, that is to say in the cold zone, the temperature curve demarcates the region. The precipitation may be sufficient to produce a covering of snow in the winter, but as the buds or shoot-apices in Chamæphytes are situated quite close to the ground, the snow protects them in winter from the severity of the unfavourable season. Similarly in warm and warm temperate zones with a dry season, the withered remains of the plants on the surface of the ground protect the buds of Chamæphytes. Here the hydrotherm figure for Jan Meyen is given.

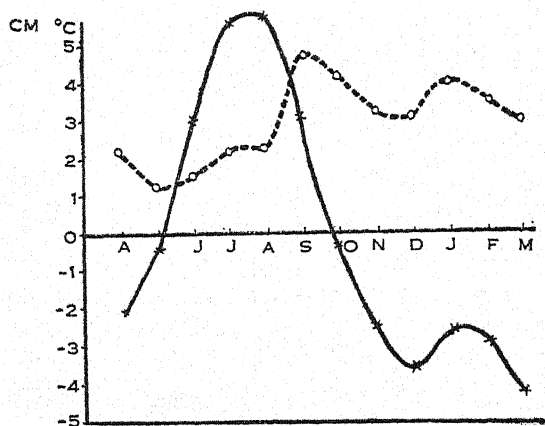


Fig. 4. Hydrotherm figure for Jan Meyen

So far no work in India has been done on Raunkiaer's system except by Borgesen⁷ who in 1929 published "Notes on the Vegetation at Dwarka". The author spent only eight days in that place and candidly admits that he only made quite short excursions of not more than a mile or so from the bungalow where he stayed. And to quote his own words "I also want to point out that even if I tried to collect all that was growing there, I am of course far from having found everything or even almost everything, this also being due to the fact that the plants growing there often were very

diminutive and therefore easy to overlook." Thus the work done in India is based upon a small number of species (28 in all) from a very restricted area.

To test Raunkiaer's theory, it was necessary to choose such areas that contained sufficient or more or less complete floristic and climatic data. Such areas are very few in India due to the absence of local floras and lack of sufficient meteorological stations.

The two hill-stations of Matheran and Mahabaleshwar afforded good opportunity to test the above theory since these places are limited in their boundaries and their flora and climatic data are fairly known.

The present work on the life-forms of the plants is based upon the following floras mentioned in the bibliography : 2, 3, 5, 6, 12, 13, 14, 16, 17, 20, 21, 22, 24, 25, 27 and 30.

1. RAUNKIAER'S LIFE-FORM SYSTEM

Raunkiaer distinguished the following ten types with their subdivisions in his life-form system (19) :—

- A. **Phanerophytes** = Ph. Plants in which the surviving buds or shoot-apices project into the air on stems more than 25 cm. above the soil level.
- I. **Megaphanerophytes** = MM. They are Ph. in which the aerial shoots rise above the soil level.
 - (1) Evergreen megaphanerophytes without bud-scales.
 - (2) Evergreen mesophanerophytes without bud-scales.
 - (3) Evergreen megaphanerophytes with bud-scales.
 - (4) Evergreen mesophanerophytes with bud-scales.
 - (5) Deciduous megaphanerophytes with bud-scales.
 - (6) Deciduous mesophanerophytes with bud-scales.
 - II. **Microphanerophytes** = M. They are Ph. in which the height of the stem varies from 2–8 m.
 - (7) Evergreen microphanerophytes without bud-scales.
 - (8) Evergreen microphanerophytes with bud-scales.
 - (9) Deciduous microphanerophytes with bud-scales.
 - III. **Nanophanerophytes** = N. They are Ph. in which the stems are under 2 m. in height.
 - (10) Evergreen nanophanerophytes without bud-scales.
 - (11) Evergreen nanophanerophytes with bud-scales.
 - (12) Deciduous nanophanerophytes with bud-scales.
 - (13) Herbaceous phanerophytes.
 - IV. (14) **Epiphytic phanerophytes** = E.
 - V. (15) **Succulent-stemmed phanerophytes** = S. They are Ph. which have succulent stems without proper foliage leaves.

- B. VI. **Chamæphytes** = Ch. Plants whose buds destined to survive the unfavourable season are situated on shoots which lie on the surface of the ground, or not more than 25 cm. above it.
- (16) Half-shrub chamæphytes (suffrutescent chamæphytes). Aerial shoots orthotropic, more or less erect, not forming cushions.
 - (17) Passive chamæphytes. Aerial shoots orthotropic, but lying down on the ground because of their own weight.
 - (18) Active chamæphytes. Aerial shoots plagiotropic and prostrate.
 - (19) Cushion-plants.
- C. VII. **Hemicryptophytes** = H. Plants whose surviving buds are situated in the soil surface, protected by the surrounding soil and by the withered remains of the plant itself.
- (20) Proto-hemicryptophytes. Without leaf-rosettes.
 - (a) Without runners. (b) With runners.
 - (21) Semi-rosette Plants. With a basal leaf-rosette and a leafy stem.
 - (a) Without runners. (b) With runners.
 - (22) Rosette Plants. With a basal leaf-rosette and a leafless or nearly leafless stem.
 - (a) Sympodial rosette-plants.
 - 1. Without runners. 2. With runners.
 - (b) Monopodial rosette-plants.
 - 1. Monopodium with foliage-leaves but no scales.
 - (a) Aerial shoots with leaves.
 - (b) Aerial shoots without leaves (only with flowers).
 - 1. Without runners. 2. With runners.
 - 2. Monopodium with both scales and foliage-leaves.
 - (a) Without runners. (b) With runners.
 - 3. Monopodium with scales only.
- D. Cryptophytes.
- VIII. **Geophytes** = G.
- (23) Rhizome geophytes.
 - (24) Stem-tuber geophytes.
 - (25) Root-tuber geophytes.
 - (26) Bulb geophytes.
 - (27) Root (bud) geophytes.
- IX. **Helophytes and Hydrophytes** = HH.
- (28) Helophytes. Vegetative shoots projecting into the air.
 - (29) Hydrophytes. Vegetative shoots submersed in water.

E. X. **Therophytes = Th.**

For statistical purpose, Raunkiaer made use of only the above ten main life-forms.

2. PHYSICAL FEATURES OF MATHERAN AND MAHABALESHWAR

Topographical Features.—Matheran is one of the hills in the Kolaba District and is situated in $18^{\circ} 58'$ latitude and $73^{\circ} 18'$ longitude, and is only 30 miles east of Bombay when measured in a straight line but 61 miles by rail.¹⁵

Matheran covers an area about 8 square miles.¹⁵ It is composed mainly of three flat-topped hill ranges, the central being the largest. It is about half a mile in breadth and extends north and south from the narrow ridge of Hart Point to Chowk Point respectively. The two other ranges stretch out on either side like wings and run more or less parallel with the main hill eastwards and westwards, connected only to the central range by narrow necks. The eastern range extends over two and a half miles from Panorama Point in the northwest to Garbut Point in the southeast, while the western or the smallest range covers only one and a half miles and extends from Porcupine Point in the north to Louisa Point in the south.¹⁵

Mahabaleshwar is one of the four hills in Javli, which is one of the eleven subdivisions of the Satara District. It lies in $17^{\circ} 51'$ north latitude and $73^{\circ} 30'$ east longitude, about 18 miles north-west of Medha, 20 miles west of Wai and 33 miles north-west of Satara. It is the chief health resort of the Bombay Presidency.

The five ranges which constitute the Mahabaleshwar Plateau are the northern ranges of the Krishna Valley, the north-western and the western ranges of the Koyna Valley, the southern ranges of the Blue Valley, and the eastern ranges of the Yenna Valley.¹³

In height Mahabaleshwar is nearly twice that of Matheran; as the latter is only 2,650 ft. above sea-level, and the former is 4,500 ft.

Geological Features.—Both Matheran and Mahabaleshwar hills consist of layers of trap of varying thickness overlaid by a light capping of iron clay or laterite. The trap is usually columnar and often accompanied by crystallised quartz, apophyllite, natrolite, stilbite, and other minerals. The iron clay or laterite is highly porous and absorbent. Its density and colour varies at different places, being red with brownish and yellowish spots in the fresh state.

The soil is made up of red clay formed by the disintegration of the laterite.

Climatic Features.—Both Matheran and Mahabaleshwar exhibit a healthy and pleasant climate and as such are health-resorts.

Their respective climates can be judged from the records given below of the mean monthly and annual rainfall, for an average of 32 years (Table II), the mean monthly and annual temperature for 1937 (Table III) and the mean monthly and annual humidity for 1937 for Mahabaleshwar only (Table IV); the corresponding humidity data for Matheran could not be obtained.

TABLE II
Mean monthly rainfall for Matheran and Mahabaleshwar in cm.

Regions	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Annual
Matheran	.22	.10	.02	.27	2.06	85.14	213.90	145.2	66.80	13.1	2.03	0.20	302.5
M'war ..	.30	.12	.33	3.63	4.44	116.23	2284.60	193.6	75.33	14.7	83.50	.86	697.7

TABLE III
Mean monthly temperature for Matheran and Mahabaleshwar in °C.

Regions	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Annual
Matheran	20.6	19.8	24.7	25.3	27.7	23.0	20.7	20.5	20.9	23.4	23.3	20.2	22.5
M'war ..	18.8	19.1	22.9	24.2	23.3	18.7	17.6	17.5	17.8	19.9	19.0	18.1	19.7

TABLE IV
Mean monthly humidity for Mahabaleshwar in percentage

Regions	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Annual
M'war ..	40	53	31	42	49	91	100	100	96	69	52	54	65

temperature point of view, the climate of Matheran may be considered very favourable. The rainfall on the other hand is not so evenly distributed. It varies from 0.1 cm. in February to 213 cm. in July. But the one thing that comes out from the study of this graph is that the annual precipitation is very high being 502 cm. which falls mostly during the period of five months, June to October. However, there is slight rain in November, December, January, February and April, and this is sufficient to keep the humidity of the atmosphere high and to favour the growth of vegetation. That this is true can be seen from the practically constant temperature graph and from the fact that the vegetative activity continues throughout the year. The principal shrub layers composed of *Strobilanthes perfoliatus* Aners, *S. Heyneanus* Nees and *Behmeria platyphylla* Talb. are always in leaf and though there are certain plants which die during one part of the year yet there are others which replace them immediately. Thus, for example, *Paracaryum caelestinum* Benth. begins to shoot during the monsoon, flowers in October and continues to do so in February and March, and finally dies down for two months. The grasses which also begin their vegetative activity during the monsoon, flower in August and September and then dry up in October and November. But these grasses are to be found only on exposed areas where the trees have been cut down. Actually under the canopy of the forest, due to very low intensity of light, no grasses are to be found, but only sedges and ferns, and these thrive right up to January or even February.

Thus it will be seen that practically throughout the year, the climatic factors favour the growth of vegetation and one cannot here speak of any unfavourable season except perhaps for a very short period during March when the rainfall is practically nil.

Mahabaleshwar has also most of the characteristics of Matheran. Like the latter, it has a very high annual precipitation and fairly high and constant temperature curve. The comparison of the hydrotherm figures 6 and 5 show the same type of curves except for the precipitation in July which is greater by 70.7 cm., due no doubt to the higher altitude of Mahabaleshwar.

In discussing the hydrotherm figures of the principal plant climates, Raunkiaer says "if the yearly precipitation is fairly high, the vegetation can well tolerate a falling off of the precipitation curve for a *single* month to below 5 cm. without causing essential change in the composition of the flora as far as life-forms are concerned."²⁹

This statement is based on the hydrotherm for Batavia.²⁹ When the hydrotherm figures for Matheran and Mahabaleshwar are considered, it is found that the rainfall of these places falls below 5 cm. for more than one month without changing the vegetation picture. Thus Raunkiaer's statement may be modified to "if the yearly precipitation is very high, the vegetation can tolerate a falling off of the precipitation even for more than a single month".

Thus it is right to say that both Matheran and Mahabaleshwar possess no dry or unfavourable season.

3. BIOLOGICAL SPECTRA FOR MATHERAN AND MAHABALESHWAR

Such regions under so favourable conditions of rainfall and temperature are bound to be characterised by a luxuriant tree growth or in the terminology of Raunkiaer they would be characterised as having Phanerophytic plant-climates. That this is so, is borne out by the biological spectra given in Table V.

From Table V, it will be seen that of the total number of plants found on these hills, 52.2% and 48.2% are made up respectively by the Phanerophytes and the Nanophanerophytes taken together. The group next in importance is the Chamæphyte which forms 17.2% and 19.6% respectively of the total number of plants. The percentages in all these groups exceed the corresponding figures in the Normal Spectrum as can be seen in Table V. Thus the plant climate may be characterised as totally Phanerophytic or Nanophanerophytic. To decide this question the following Raunkiaer's formula may be applied here.

$$\frac{1_a}{n_a} = \frac{1_b}{n_b}$$

where 1_a stands for the percentage number of a life-form in the spectrum of the local flora and n_a for the percentage of the same life-form in the Normal Spectrum, 1_b for the percentage number of the second life-form in the spectrum of the local flora and n_b for the percentage of the same life-form in the Normal Spectrum. On substituting the respective numbers of the two life-forms, Phanerophytes and Nanophanerophytes for each place, we get the following result.

$$\frac{31.1}{28.0} = \frac{21.1}{15.0} \text{ for Matheran}$$

$$\text{or} \quad 1.1 = 1.4$$

$$\text{and} \quad \frac{28.6}{28.0} = \frac{19.6}{15.0} \text{ for Mahabaleshwar}$$

$$\text{or} \quad 1.0 = 1.3$$

Thus in the former as well as in the latter, the Phanerophytes are less than the Nanophanerophytes. However it is possible to combine these two groups of life-forms into one and compare their percentage with their corresponding percentage in the Normal Spectrum. By doing so we decidedly get a Phanerophytic climate, the percentage number exceeding the corresponding percentage in the Normal Spectrum by 9% and 5% respectively.

This combination of the two life-forms is justifiable when we consider the fact that we have not divided the Phanerophytes into Micro-, Meso-, and Mega-phanerophytes, and also the fact that

TABLE V

Regions	Number of Species	The percentage distribution of the species among the life-forms									
		Ph	N	Ch	H	G	HH	Th	L	P	E
Matheran ..	361	31.1	21.1	17.2	2.2	4.2	0.6	10.5	11.1	0.6	1.4
M'war ..	469	28.6	19.6	19.6	3.4	3.2	0.6	13.2	7.9	0.9	3.0
Normal Spectra ..	1000	28.0	15.0	9.0	26.0	4.0	2.0	13.0	3.0

the average height of the Phanerophytes in Matheran and Mahabaleshwar does not exceed 60-70 ft., that is to say there are no Mega-phanerophytes. Combining these two life-forms into one group has moreover been done by Raunkiaer while comparing the biological spectra of tropical places like Seychelles, St. Thomas and St. Jan and Aden²⁹ and also by Allan in his study of New Zealand.¹ See Tables VI and VII.

TABLE VI

Regions	No. of Species	The percentage distribution of the species among the life-forms									
		S.	E.	MM.	M.	N.	Ch.	H.	G.	HH.	Th.
Seychelles	258	1	3	10	23	24	6	12	3	2	16
St. Thomas & St. Jan	904	2	1	5	23	30	12	9	3	1	14
Aden	176	1	7	26	27	19	3	..	17
Normal Spectrum	400	1	3	6	17	20	9	27	3	1	13

TABLE VII

General Comparison of Normal and New Zealand Biological Spectra

Regions	No. of Species	S.	E.	MM.	M.	N.	Ch.	H.	G.	HH.	Th.
Normal Spectrum	1000	2	3	8	18	15	9	26	4	2	13
New Zealand	1584	..	1	3	12	17	14	39	5	3	6
Normal Spectrum					Ph. 46		Ch. 9	H. 26		Cr. 6	Th. 13
New Zealand					33		14	39		8	6

The above study of the Biological Spectra of Matheran and Mahabaleshwar in relation to their hydrotherm figures bring out clearly that both have a Phanerophytic plant-climate which is characteristic of the tropical regions. Within the regions of Phanerophytes there are of course subdivisions which are characterised by their percentage of Mega-, Meso-, and Micro-phanerophytes. In short as said by Raunkiaer, "the characteristics of all tropical lands in which the precipitation is not too small, the centre of gravity in the biological spectrum is amongst the Phanerophytes. This is not

so as in any other climate. The tropical climate which is not too dry is expressed as a plant climate, a 'Phanerophytic Climate'.²⁹

This resemblance in their plant-climate is further accentuated by similarity in their types of forests. Both belong to the Sub-tropical wet-evergreen hill forests¹¹ and their climatic-climax vegetation seem to be alike.

In spite of the above resemblances, the two floras differ in certain respects for example Mahabaleshwar has a greater number of species than Matheran: 469 as against 361. This may be partly accounted for by the much greater area of the former (whereas Mahabaleshwar is 49,258 acres, Matheran is 5,000 acres only). Another point which may be emphasized is the percentage of Epiphytes which in Mahabaleshwar amounts to as much as in the Normal Spectrum, namely 3%. In Matheran, however, it amounts to 1.4% only. This might be due to lower precipitation, lower altitude, and lower density of the trees as can be seen from the following table.

TABLE VIII

	Mahabaleshwar	Matheran
Area	49258 acres	5000 acres
Altitude above sea-level	4710 ft.	2650 ft.
Density per acre.	1312	994
Precipitation in 1940	697.7 cm.	302.5 cm.

These figures are taken from the Working Plans^{37, 38} of the respective places which were made in 1935 and 1932.

SUMMARY AND CONCLUSIONS

1. As no study was done in India on the basis of Raunkiaer's Life-Form System, the present work was undertaken along with about five other places of the Deccan.

2. The present bio-statistical study was made possible due to the fact that both Matheran and Mahabaleshwar are limited in their boundaries and their floristic and climatic data are fairly known and complete.

3. The biological spectra for Matheran and Mahabaleshwar were then studied in correlation with their topographical and climatic features, and were compared with the spectra of other tropical lands and then the following conclusions are drawn:—

(a) That both the places show decidedly a Phanerophytic plant-climate with 52 and 48 per cent. respectively of Phanerophytes.

(b) That the method of representing plant-climates by biological spectra has proved satisfactory as far as the present study is concerned.

ACKNOWLEDGEMENTS

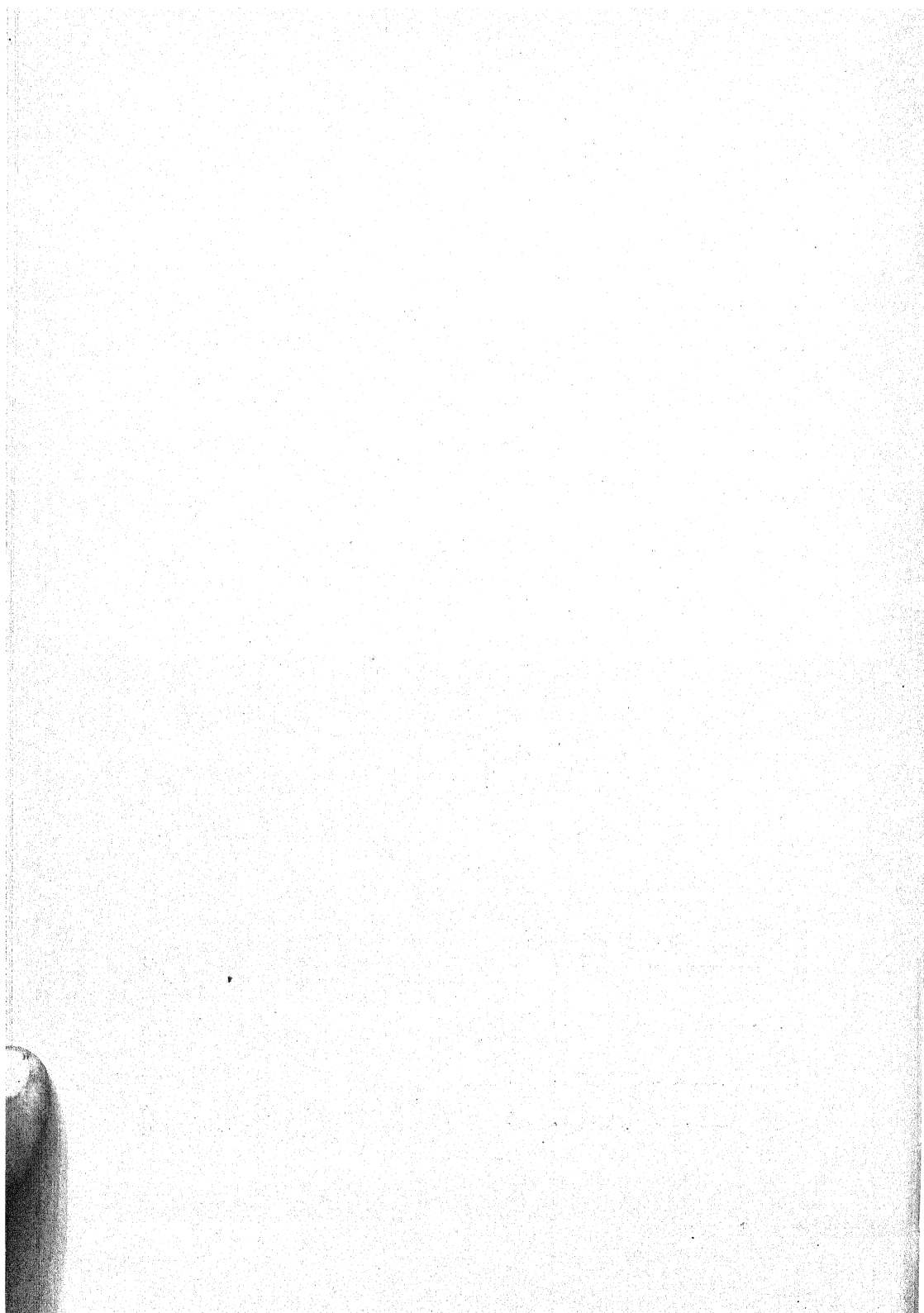
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EFFECT OF LIGHT INTENSITY AND TEMPERATURE ON THE GROWTH OF AZOLLA FILICULOIDES

BY AHMAD, GHIAS-UD-DIN, B.SC. (AGRI.) (PB.), B.SC. (LOND.),
M.SC. (CALIF.)

Assistant Professor of Botany

Punjab Agricultural College, and Research Institute, Lyallpur

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1. INTRODUCTION

In 1905, F. F. Blackman's² classical paper on "Optima and limiting factors," an outgrowth of Leibig's "Law of minimum" was published.

It has been successfully shown by many workers that Blackman's theory is not of general applicability. There is little doubt that conditions exist where more than one factor determine the rate of a physiological process in a plant.

The results reported in this paper, along with other previous works, which will be mentioned briefly in the review of literature, prove that "Law of limiting factors" as postulated by Blackman, must now be understood as modified by Harder⁶ and Lundegardh.⁸

Our present ideas on the subject can be expressed very aptly in the unpublished words of Prof. A. R. Davis: "A physiological process may be limited by one or more factors the degree of limitation effected being determined by the relative dominance of factor; and as a corollary of this, where a process is limited by two or more factors, the nature and degree of the effect produced is a function of limiting factor inter-relationship." This is Harder's⁶ idea, but stated in more general terms, and in fact applicable to all physiological processes.

From the title of this paper it is clear that this problem, when reduced to simplest terms, really comes to finding the effect of two limiting factors and their inter-relationship upon the growth of *Azolla* sp.

Only two factors, light and temperature were varied. The work was carried out under controlled laboratory conditions of light, temperature, air, culture solution, etc. Thus an environment was established which can be duplicated at will, and made it possible in turn to duplicate the results. Such a controlled environment permits the isolation of a single variable and a quantitative study of diverse physiological phenomena.

Azolla filiculoides is a common water fern in California. It belongs to the family Salviniaceæ. The leaves are arranged in two rows, each leaf is two lobed, and there are true roots. The sporophyte branches extensively and these lateral shoots readily separate, in this way the plants multiply with extraordinary rapidity.

The reason for the selection of *Azolla* sp. for this work are: firstly it is available throughout the year, secondly, it is very conveniently centrifuged in order to find the fresh weight, which was made the criterion of the relative growth in the experiment. The plants are easy to handle and are not injured, provided due care is taken.

2. REVIEW OF LITERATURE

A study of the inter-relation of conditioning factors, while working on photosynthesis led Blackman² to formulate his 'Principle of Limiting Factors' which he stated in the following axiom: "When a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the slowest factor." This was supported by the work of Miss Mathæi,¹⁰ Blackman and Smith³ and others.

Boysen Jensen,⁴ Lundegardh,⁸ Harder,⁶ and Warburg¹⁵ submitted the theory to a good deal of destructive criticism.

There are two main points of difference between the viewpoints of Blackman and Harder, viz., First, according to Blackman, there is a sudden change in the direction of the ascending curve to a horizontal one, when the limiting factor comes into operation, while Harder has shown that the curves are extremely regular and there is no sudden change of the ascending curve to a horizontal phase. Secondly, Harder believes, as opposed to Blackman, that the rate of a process is not governed by one factor and that alone, but is also conditioned by the intensity of others present in relative minimum. It will be seen from the results reported in this paper that Harder's contention is supported by the work on *Azolla* sp.

Lubimenko⁹ found that in heliophilous plants the rate of the accumulation of elaborated material was increased with increase in the light intensity upto an optimum point, and that any increase beyond the optimum resulted in a decrease in the rate. Helio-phobous plants had the optimum at a much lower point than the heliophilous types.

Popp¹² showed that the various parts of the spectrum have very different effects upon the growth and reproduction of plants. His results, as a whole, indicate that, while ultra-violet rays are not indispensable, the blue-violet end of the spectrum is necessary for normal, vigorous growth of plants. His paper is of particular importance for those who have to use artificial light in physiological experiments.

Porter¹³ showed that light intensity seems to have a regulatory effect on the average amount of chlorophyll per unit leaf area. A light intensity averaging 1139.9 foot-candles daily during the growth of the tomato plants had a greater effect in promoting chlorophyll formation, fruit formation and photosynthetic efficiency than light of a daily average of 583.1 foot-candles, this in turn had a similar greater effect than a daily average light intensity of 216.0 foot candles.

The results under discussion in this paper show that light intensity within certain range does increase the growth of *Azolla* and beyond that range, it has an adverse effect on the chloroplasts of the plants and the plants suffer from too much illumination.

Arthur, Guthrie and Newell,¹ working with thirty different species of plants, and light intensities of 350, 450, 760, 800, 1200, and 1400 foot-candles came to the conclusion that the "time factor" was important. The maximum carbohydrate increase was reached at higher light intensities in shorter length of the day, as compared with the low intensities of light. The observations made during the work under report support the views of Arthur and his co-workers and will be discussed in greater detail in the later part of the paper under the heading of 'time factor'.

Plätzer¹¹ defines the compensation point as the light intensity at which the respiratory and photosynthetic activities compensate each other and the gaseous exchange is consequently zero. The position of the compensation point of a plant in regard to temperature is naturally of great importance to the life of the plant and its relation to the environment. As this point is dependent upon the rate of respiration, it varies greatly in different plants. It appears that the compensation point is lowered with decreasing temperature.

In the light of this work, the paper under report brings out very interesting data and the significance of compensation point in *Azolla* sp. will be discussed below in some detail.

Whereas the paper under report fully supports the criticism of Singh and Lal¹⁴ of "Limitations of Blackman's Law of Limiting Factors", it also upholds Harder's "Concept of relative minimum as applied to photosynthesis".

3. THE EXPERIMENTAL TECHNIQUE

Healthy and uniform *Azolla* plants were selected from a tank. The plants were grown in one quarter strength Hoagland solution as used by Hoagland & Broyer,⁷ in pyrex glass beakers. The solution

was changed once a week. The beakers with plants were kept at a constant temperature in water-baths, the temperature of which was kept constant by electric mercury thermo-regulators.

The light was supplied by gas filled Mazda lamps as recommended by Davis, A. R. & Hoagland, D. R.,⁵ varying from 25 W to 750 W, for 16 hours daily. A Hartford electric time switch of 220 volt 50 ampere capacity was employed for controlling the daily illumination period. It is clear that the whole arrangement was automatic.

Fresh weight of the plants was taken as the criterion of growth. In order to find the fresh weight at the beginning and at the end of each experiment, the plants were put in cheese cloth, moistened and centrifuged at the rate of 400 revolutions per minute for five minutes. The plants were again dipped in water and after centrifuging, weighed, till the last two weights agreed. This method is due to Hoagland and Broyer.²

The Temperature of the Plant.—The temperature of the baths was controlled by mercury thermo-regulators and the beaker containing plants were put inside vitex pipes, two feet in height and eight inches in diameter. The Mazda electric bulbs of different watts were placed on the upper side of the pipes in dome-shaped covers.

The bulbs gave out heat besides light, which raised the temperature of the plants. The vitex pipes had series of holes towards the bottom and top, through which water and air could freely circulate and thus keep the temperature of the plants down by keeping the temperature of the culture solution in the beakers to that of the bath. The temperature of the water in the bath and the plants was both noted.

The Light intensity.—Throughout the experiment, gas-filled Mazda electric bulbs were used and their light intensity measured with single phototronic cell, in foot-candles, under experimental conditions.

The Procedure.—Four lots of two grams each fresh weight of *Azolla* plants, were exposed to various intensities of light at a constant temperature for one week for sixteen hours daily. The temperature of the bath was changed by 5°C. every week. At the end of the week, the fresh weight was taken and thus the gain or loss in weight was noted.

4. THE DATA

Tables I-IV indicate the influence of temperature on the yield of *Azolla* sp., daily exposure period was sixteen hours, and the duration of each experiment was one week.

TABLE I

Illumination 35.36 Foot-Candles

Temperature of the		Fresh weight		Percent- age of gain or loss in one week	Remarks
Bath $\pm 0.1^{\circ}$ C.	Plants $\pm 0.5^{\circ}$ C.	Initial	After one week		
		gms.	gms.		
5°	6.5°	2.00	2.39	19.5	Upto $21^{\circ} \pm 0.5^{\circ}$ C. only, in this light intensity an in- crease in fresh weight took place and at higher temperatures there was a loss of weight.
10°	11°	2.00	3.12	56.0	
15°	16°	2.00	3.23	61.5	
20°	21°	2.00	3.28	64.0	
25°	25.5°	2.00	1.74	-18.0	
30°	30°	2.00	1.63	-18.5	
35°	35°	2.00	1.46	-27.0	

TABLE II

Illumination 71.85 Foot-Candles

Temperature of the		Fresh weight		Percent- age of gain or loss in one week	Remarks
Bath $\pm 0.1^{\circ}$ C.	Plants $\pm 0.5^{\circ}$ C.	Initial	After one week		
		gms.	gms.		
5°	7°	2.00	2.45	22.5	In this light in- tensity there was an increase in weight upto $30.5 \pm 0.5^{\circ}$ C. At higher temperatures there was a loss of weight.
10°	11°	2.00	3.82	91.0	
15°	16.5°	2.00	4.21	110.5	
20°	21.5°	2.00	4.53	129.0	
25°	26°	2.00	2.65	32.5	
30°	30.5°	2.00	2.44	22.0	
35°	35°	2.00	1.89	- 5.3	

TABLE III

Illumination 122.5 Foot-Candles

Temperature of the		Fresh weight		Percent- age of gain or loss in one week	Remarks
Bath $\pm 0.1^{\circ}$ C.	Plants $\pm 0.5^{\circ}$ C.	Initial	After one week		
		gms.	gms.		
5°	7.5°	2.00	2.56	28.1	In this light intensity there was an increase in weight even at 35.5° C.
10°	12°	2.00	4.67	133.5	
15°	17°	2.00	5.28	164.0	
20°	22°	2.00	5.93	196.5	
25°	26.5°	2.00	3.80	90.0	
30°	31°	2.00	3.31	65.5	
35°	35.5°	2.00	2.07	3.3	

TABLE IV

Illumination 191.3 Foot-Candles

Temperature of the		Fresh weight		Percent- age of gain or loss in one week	Remarks
Bath $\pm 0.1^{\circ}$ C.	Plants $\pm 0.5^{\circ}$ C.	Initial	After one week		
		gms.	gms.		
5°	9°	2.00	3.07	53.5	In this light intensity there was a greater increase in weight at 35° C. as compared to all the previous cases.
10°	13.5°	2.00	5.79	189.5	
15°	16.5°	2.00	6.48	224.0	
20°	22°	2.00	7.09	254.0	
25°	27.5°	2.00	4.62	130.0	
30°	31.5°	2.00	4.03	101.5	
35°	36°	2.00	2.18	9.0	

TABLE V
Showing the Combined Data of Tables I-IV

Light intensity in foot- candles	Percentage of gain or loss in fresh weight of the plants in one week at various temperatures							Remarks
	Temperature of the water bath $\pm 0.1^{\circ}$ C.							
	5° C.	10° C.	15° C.	20° C.	25° C.	30° C.	35° C.	
35.36 F.C.	19.5	56.0	61.5	64.0	-18.0	-18.5	-27.0	For the temperature of the plants please refer to Tables I-IV.
71.85 F.C.	22.5	91.0	110.5	129.0	32.5	22.0	- 5.3	
122.5 F.C.	28.0	133.5	164.0	196.5	90.0	65.5	3.3	
191.3 F.C.	53.5	189.5	224.0	254.0	131.0	101.5	9.0	

5. DISCUSSION

Growth is properly speaking an expression of the metabolism of the organism. It is difficult to describe it more aptly than to use F. F. Blackman's phrase that the growth is the finished product of the metabolic loome. Prof. A. R. Davis expresses the same idea with the help of a simple equation (unpublished) as follows :—

$$Y = E_p - E_r$$

Where Y represents the growth, E_p and E_r represent photo-synthetic and respiratory energies respectively. It is clear that the value of Y will depend upon the gain of E_p over E_r .

Table I.—If we refer to Table I, we find that the low intensity of light, viz., 35.36 foot-candles used in the experiment was conducive to growth only up to about 22°C. (the temperature of the plants), but at higher temperature namely 25°C. etc., there was a loss of weight to the extent of 18–27 per cent.

This means that the plant gained less in photosynthesis and lost more due to respiration at 25°C. At lower temperatures than 22°C., the plant gained in weight even in the low intensity of 35.36 foot-candles. It seems that the loss in weight due to respiration was almost constant from 25–30°C. but it increased at 35°C. with the result that the plant lost 27 per cent. in fresh weight.

The data of Table I throws light on the fact that low intensity of light helps in growth at lower temperatures, but at higher temperatures it becomes a limiting factor for growth due to the increased rate of respiration.

Table II.—The data of Table II brings out the same points. With the increase in the light intensity to 71.85 F.C., growth takes place even at 30°C., due to increase in photosynthesis, but at 35°C. the rate of respiration increases and photosynthesis being unable to keep pace with it, results in the loss of weight.

Tables III and IV.—The data of Tables III and IV indicates that the increased light intensity of 122.5 F.C., and 191.3 F.C. is able not only to compensate the loss in weight in respiration but also maintain growth even at 35°C.

Table V.—Table V, summarises the data of the previous one to four tables. If the table is read from left to right, it becomes evident that at each intensity of light, the rate of growth goes on increasing up to about 22°C. and after that there is a decrease in the rate of growth. On the other hand if the table is read from the top to the bottom, it can be seen that with the increase in the light intensity at all temperatures (5°C.–35°C.), the rate of growth increases. It is, therefore, evident that the rate of growth under these circumstances is dependent both upon the intensity of light and temperature.

In the mathematical equation :—

$$Y = E_p - E_r$$

If $E_p = E_r$ then Y becomes zero. It is evident, as already discussed that the growth represented by Y, depends upon the value of (E_p en E_r). It has, however, to be emphasised that the

photo-synthesis and respiration are both affected by a change in temperature and light intensities to different degrees.

The rate of respiration relatively decreases much more at lower temperatures than the rate of photosynthesis, with the result that growth at lower temperature is possible than at higher temperature even in the low light intensities, as is clear from Table V.

With the increase in temperature, photosynthesis after reaching its climax does not increase further, but respiration goes on increasing in a geometrical progression, with the result, that even in the best illumination, with the increase in temperature, a decrease in weight takes place.

Compensation point.—If the light intensity is gradually increased from complete darkness, a point will be reached where the gaseous exchange with the atmosphere will become zero. If this state of affairs continues for a long time, then the plant will neither gain nor lose in weight. This point has been termed the "Compensation Point" by Plätzer.¹¹

The position of the compensation point of a plant in regard to temperature is naturally of great importance to the life of the plant and its relation to the environment.

The Inter-relation of the light and temperature factors.—The results of Tables I-IV are graphically represented in Figs. 1 and 2

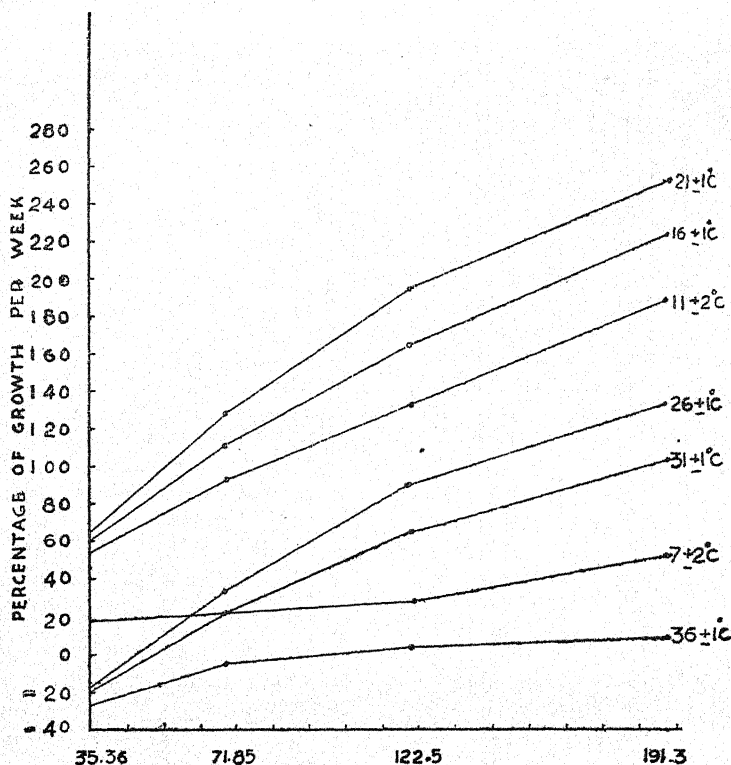


Fig. 1. Light intensity in Foot-candles. Effect of change in light intensity on the rate of growth of *Azolla* sp. at different temperature,

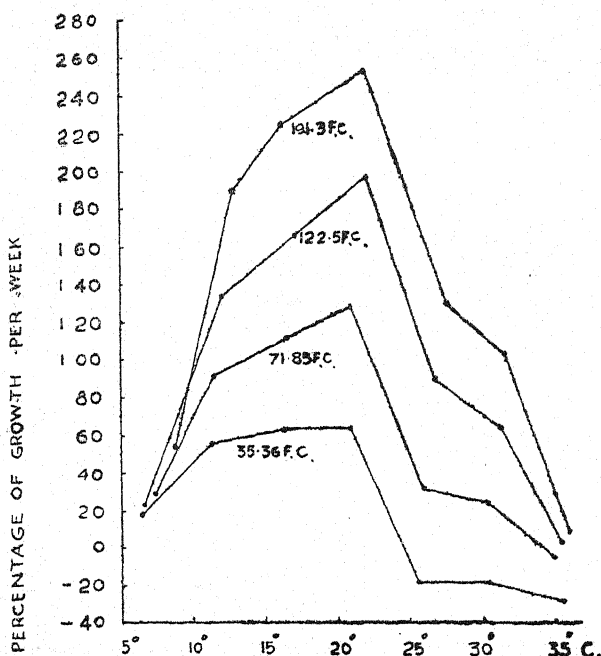


Fig. 2. Temperature of the Plants. Effect of change in temperature on the rate of growth of *Azolla* sp. in different light intensities.

in the text. It may be mentioned here that the temperature of the bath was kept constant throughout the week but the temperature of the plants was higher during the period of illumination and this higher temperature is also shown in Tables I-IV.

It seems clear from the data presented in this paper that at least two primary limiting variables influence the form of the ascending portion of the curve, temperature and light, and that the influence exerted ranges from the dominance of one to the dominance of the other. Throughout the range, however, the actual growth becomes a function of limiting factor inter-relationship.

The point worthy of note is that in Fig. 1, the curves are almost regular in appearance and there is no sudden change of the ascending curve to a horizontal phase, contrary to the findings of Blackman. It may, however, be pointed out that the curve for $7^{\circ} \pm 2^{\circ} \text{C.}$ in Fig. 1, shows a jump at 191.3 foot-candles illumination, for the reason that in this high light intensity the temperature of the plants was 4°C. higher than the temperature of the water-bath (*vide* Table IV), hence the jump. At higher than 10°C. temperatures of the water-bath, the difference in the temperatures of the plants and the water-bath in all the light intensities was not so great as at 5°C. In lower than 191.3 foot-candles illumination, the difference in the temperature of the water-bath and the plants was almost uniform

($\pm 1.5^{\circ}\text{C.}$), hence it may confidently be stated that the data presented in Table V and the curves in Fig. 1, support Harder's contention rather than Blackman's hypothesis.

From Fig. 2, it is clear that in *Azolla* sp., the peak of growth in all cases is reached at about 22°C. and this maximum is constant in the low, medium and high intensities of light. At higher temperatures than 22°C. the behaviour of the plant is different in different light intensities, according as these influence the rate of photosynthesis and respiration.

The Time Factor.—

TABLE VI

Growth of Azolla sp., at 10°C. during One Week

Light Intensity	Fresh weight		Percent- age of increase in fresh weight in one week	Period of illumina- tion per day	Remarks
	Initial	After one week			
	gms.	gms.		hrs.	
100 W (35-36 F.C.)	2.00	3.37	68.5	24	A new bulb.
100 W	2.00	3.05	52.5	16	This was an old bulb.
40 W (9-17 F.C.)	2.00	2.18	9	16	
25 W (5 F.C.)	2.00	2.08	4	16	

From Table VI, it is seen that in the low light intensity of 35.36 foot-candles (100 Watts.), when the period of illumination was increased from 16 hours to 24 hours daily at $11 \pm 0.5^{\circ}\text{C.}$ (the temperature of the plants) for one week, it resulted in the increased growth. On the other hand, when the plants were exposed to 191.3 foot-candles light intensity for 24 hours daily at $13.5 \pm 0.5^{\circ}\text{C.}$ (the temperature of the plants) for one week, the plants looked scorched due to this high light intensity, while this very intensity of light, when working for 16 hours daily had produced excellent results (*vide* Table IV). This brings out the importance of the time factor for different light intensities. With time the maximum rate of growth shifts besides to a lower temperature to a lower intensity of light as well.

Internal Factors.—In addition to the chlorophyll factor there is the important protoplasmic factor which plays a very important part and is influenced particularly by changes in temperature. Singh, B. N., and Lal, K. N.¹⁴ have rightly laid emphasis on the point that any relationship that is traced between the external factors

alone without taking into consideration the internal changes that take place in the process as well as the intensities in which the external factors reach the internal tissues, will not hold good under all circumstances.

Possibility of the duplication of experimental data.—From Table I, it is clear that in 35·36 F.C. illumination, at $11 \pm 0.5^{\circ}$ C. a 56 per cent. increase in fresh weight occurred. The data in Table VI, which was obtained several weeks later, shows an increase of 52·5 per cent. under the same circumstances. The difference of 3·5 per cent. less is probably due to the fact that the bulb was not a new one and was giving out a lower illumination than 35·36 F.C. It is, however, unfortunate that the exact light intensity of this bulb was not measured. To obtain result so approximately close to each other, clearly shows the practical possibility of the duplication of experimental data, as already suggested and shown by Davis, A. R., and Hoagland, D.R.⁵ It is evident, that conditions can be so standardized that one variable will always bear the same relation to another.

Light intensity and root development.—One observation in this connection is of special interest. In low light intensities, the root system did not develop well and the older roots fell off. When the light intensity was increased, the development of the roots improved. This observation shows the importance of light on the root development of *Azolla* sp.

Economic importance of this type of investigation.—The tomato stands foremost among the several vegetable plants which are cultivated as green house crops in the various parts of the U.S.A. and in Europe. During winter months and in cloudy weather, the short length of the day and the low intensity of light seriously affects the yield of crops and hence the profits of the green house farmers are depleted. This factor becomes so serious in certain localities that vegetable production in green houses cannot be carried on economically. The results reported in this paper, if further amplified according to a particular problem, will prove of great advantage in such cases.

It further brings out the importance of the inter-relationship of the temperature and light intensity in the growth of the plants.

6. SUMMARY AND CONCLUSIONS

1. An experimental technique for the study of the influence of temperature and light intensity upon the growth of *Azolla* sp. is described.
2. The growth of *Azolla* sp. in the culture solution was very good, provided light and temperature were not acting as limiting factors.
3. No organic matter or extracts from yeast, peat, etc., were added to the culture solution. It means that the absence of

so-called "Auximone" did not make any difference in the growth of *Azolla* sp.

4. In lower intensities of light, root system showed very poor development, in fact the older roots fell off from the plants, as illumination was increased, a great improvement in the development of roots was noticed.

5. From Table V, in the text, it is seen that light and temperature both act as limiting factors simultaneously, since an increase in either of these factors favourably influences the growth of *Azolla* sp. within a limited range.

6. At the low intensities of light and temperatures for instance in 35·36 foot-candles at 6·5°C. it was temperature that was present in most nearly minimal amount and not light. This conclusion is drawn from the fact that doubling the light intensity resulted in much less increase in growth, whereas increasing the temperature from 6·5° C. to 11° C. showed much more increase over the former temperature in the same illumination.

7. Increase in the factor which is present in most nearly minimal amount results in the most favourable increase.

8. The curves obtained are almost regular and do not show a sharp bend, as postulated by Blackman.

9. The maximum growth of *Azolla* sp. took place at $22^{\circ} \pm 1^{\circ}$ C., in low, medium and high intensities of light.

10. The importance of the internal factors, time factor, the inter-relationship of the factors and compensation point is emphasized.

11. The practical possibility of the duplication of experimental data is shown.

12. The possibility of the application of these results to supplement light in the green houses during winter months and in cloudy weather, for vegetable culture like tomato, etc., is mentioned.

13. In conclusion the results reported in the paper fully substantiate the previous works of Harder, Lundergardh, and Warburg.

14. During the period of the author's leave from the Punjab Agricultural College, Lyallpur, he had the privilege of working under Prof. A. R. Davis at Berkeley, the University of California. The problem was suggested by him and the work was carried out under his kind and excellent guidance for which the author is most grateful. He was further kind enough to allow the citation of his unpublished opinion on the principle of limiting factors and his growth equation, which were most helpful in explaining the data obtained.

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REVIEW

Norman C. Fasset.—A Manual of Aquatic Plants. Pp. 382.
McGraw Hill Publishing Co., Ltd., London, 1940, 28/-.

THE aim of this book is to facilitate the identification of water-plants by providing tolerably large size figures of the plants. The textual portion, according to the author himself, "is essentially a set of directions for looking at the pictures". The book can rightly be termed as a picture book of aquatic plants, and all the diagrams are extremely clear. Part I of the book, extending over 33 pages is a general key, with the help of which the name of a plant can be arrived at. The key is arranged in such a fashion that most plants can be identified with certainty from sterile material itself. Part II of the book gives a descriptive treatment of families, genera, and species in the regular systematic fashion, and is intended to be referred to after a plant has been identified with the help of the general key in Part I. The aquatic plants included in the book are all those that can be seen with the naked eye. So the list includes a few algæ as well. A number of algæ which require the use of a microscope for definite identification, although those are visible to the naked eye, have been excluded. The greater portion of the book naturally deals with angiospermous aquatics, and the description of these is exhaustive. There is an important appendix showing how the aquatic plants are utilised by birds and mammals (wild life) and also a separate list which gives the relation of plants to fish. This manual should prove extremely useful to students and all others who have to collect and identify aquatic plants.

V. S. R.

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RECENT WORK ON THE TYPES OF EMBRYO-SACS IN ANGIOSPERMS— A CRITICAL REVIEW

BY P. MAHESHWARI

Dacca University

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INTRODUCTION

SINCE the publication of my former article, entitled "A critical review of the types of embryo-sacs in Angiosperms" (*New Phytologist*, 1937) a considerable amount of work has been done in this field which has prompted me to return to the subject and write the present essay. Although this is mainly concerned with the work

of the past 4 years, some earlier publications have also been included where it seemed desirable to do so for purposes of comparison.

The various modes of embryo-sac development known at present may be classified under the following major types :—

MONOSPORIC	..	<i>Normal-type</i> <i>Oenothera-type</i>
BISPORIC	..	<i>Allium-type</i>
TETRASPORIC	..	<i>Peperomia-type</i> <i>Fritillaria-type</i> <i>Plumbagella-type</i> <i>Plumbago-type</i> <i>Adoxa-type</i>



















































































NORMAL-TYPE

Terminology and usual course of development.—In the introduction to my last paper I stressed the need for a uniform terminology in describing the development of the embryo-sac. Recent literature is nevertheless not entirely free from ambiguity.

Thus, Billings (1937, p. 307) wrongly uses the term embryo-sac mother cell for what is clearly the functioning megaspore. Cave (1939) writes that in *Leucocoryne* "the macrospore mother cell divides to give rise to two macrospores" (obviously the dyad cells). Walker (1938a, p. 594) says that the polar nuclei fuse before fertilisation to form the primary endosperm nucleus (really the secondary nucleus, because the endosperm nucleus is a product of triple fusion). Sometimes the polar nuclei have themselves been called endosperm nuclei (Stover, 1937).

Greater confusion is seen with regard to the names applied to the products of reduction division of the megaspore mother cell than to any other stage. As early as 1907, Pace remarked "It is unfortunate that there is no definite name for the two cells resulting from the division of the megaspore mother cell." They have been called "daughter cells", "megaspores" and "derivatives of the embryo-sac mother cell." Of these terms, the first and the last are vague and the second is used incorrectly because the process of reduction division is not yet complete, and megaspores can be said to have been formed only after the hetero- and homotypic divisions have taken place. As pointed out by me earlier (Maheshwari, 1937) it seems that "dyad cells" would be the least objectionable. Johansen (1940) calls them "secondary megasporocytes".

A row of 3 cells is frequently met with after reduction but all the cells are not megaspores—one must be an undivided dyad cell. Members of the family, *Amaranthaceae* investigated by Kajale (1940), show all transitions from the normal row of four cells to a row of 3 cells. This is due to an irregularity in the behaviour of the micropylar dyad cell; sometimes it divides as usual like the chalazal dyad cell, in other cases it divides late or not at all. In

Type	Mother cell	I Division	II Division	III Division	IV Division	V Division	Mature embryo-sac
Monosporic 8-nucleate <i>Normal-type</i>							
Monosporic 4-nucleate <i>Oenothera-type</i>							
Bisporic 8-nucleate <i>Allium-type</i>							
Tetrasporic 16-nucleate <i>Peperomia pellucida</i> -form							
Tetrasporic 16-nucleate <i>Peperomia hispida</i> -form							
Tetrasporic 16-nucleate <i>Gunnera</i> -form							
Tetrasporic 16-nucleate <i>Penaea</i> -form							
Tetrasporic 16-nucleate <i>Pyrethrum</i> -form							
Tetrasporic 16-nucleate <i>Drusa</i> -form							
Tetrasporic 16-nucleate <i>Acalypha indica</i> -form							
Tetrasporic 8-nucleate <i>Fritillaria</i> -type							
Tetrasporic 4-nucleate <i>Plumbagella</i> -type							
Tetrasporic 8-nucleate <i>Plumbago</i> -type							
Tetrasporic 8-nucleate <i>Adoxa</i> -type							

some plants nuclear division occurs but no cell-wall is laid down between the daughter nuclei.

The megaspores in the tetrad may be arranged in various ways. The commonest form is the linear one, in which all the four cells lie in the same row. The "T"-shaped arrangement is less common but quite frequent, and if both the cells at the micropylar end are not visible in the same section, one may easily get the impression of having seen only 3 cells. An arrangement like that of the inverted "L" is rare and has been reported only as an abnormality, as in *Tacca viridis* (Paetow, 1931), *Styrax officinalis* (Copeland, 1938), *Cyathula tomentosa*¹ (Kajale, 1940) and *Desmodium gangeticum* (Pantulu, 1941). Occasionally a tetrahedral arrangement may be seen, as in *Vallisneria spiralis* (Witmer, 1937) and *Enalus acoroides* (Kausik, 1940). In *Daphne laureola*, Fuchs (1938, Fig. 20) shows an isobilateral tetrad of megaspores.

Occasionally all the four megaspores may start developing, as in *Putoria* (Fagerlind, 1936), but usually three are suppressed and only one grows further. This is the functioning one. All kinds of exceptions are, however, on record and it seems unnecessary to cite particular instances; a very full account of these is given in the two volumes of Schnarf (1929, 1931).

The 2 nuclei formed from the first division of the functioning megaspore may be designated as the primary micropylar and primary chalazal. Although they move to the two poles, they should not be called "polar nuclei," since this term is more frequently used to designate the 2 free nuclei of the mature embryo-sac.

Normally there are two further divisions to give rise to an 8-nucleate stage, composed of a micropylar quartet and a chalazal quartet. The mature embryo-sac is composed of an egg apparatus (egg and 2 synergids), 3 antipodal cells or nuclei, and the two polar nuclei. The differentiation of cells at the two ends of the embryo-sac may not proceed simultaneously. Usually the cells of the egg apparatus are the first to be organised but in the *Amaranthaceae* (Kajale, 1940) the antipodals differentiate first, this being specially well observed in *Celosia argentea* and *Allmania nodiflora*.

Starch grains in the embryo-sac are frequently met with [see Dahlgren (1927, 1939) for a résumé of the subject]. Sometimes the starch grains in the embryo-sac get incorporated in the egg, as in *Euchlaena mexicana* (Cooper, 1938), *Portulaca* (Cooper, 1940) and some other plants. In *Acacia baileyana* (Newman, 1934) starch is present even in the synergids and antipodals and the author suspects that the centrosomes figured in the egg cell by some older authors may really be only starch grains.

Venkateswarlu (1937) reports a peculiar pale-staining body in the megaspore mother cell of *Sonneratia apetala* which persists even up to the mature embryo-sac stage. Karsten (1891) also figured

¹ See also Maheshwari (1941).

similar bodies and described them as oily looking. This is confirmed by Mauritzon (1939) who calls them fat bodies and gives some details about their position and behaviour in *Sonneratia acida*.

Embryo-sacs with 8 nuclei but peculiar organisation.—In *Eragrostis cilianensis* (Stover, 1937), after the 8 nuclei have been formed in the normal fashion, the 4 chalazal ones are said to become separated from the rest of the embryo-sac as antipodal cells; one or two of these divide immediately so that there are commonly 5–6 antipodals at the time of fertilisation. The 4 nuclei at the micropylar end of the sac are said to give rise to the egg, one synergid and 2 polar nuclei. The author adds that the “organisation of the embryo-sac is a new type not only for grasses but for all plants”. Unfortunately the figures are not convincing and a reinvestigation of the plant is necessary before his interpretations can be accepted. It is very unlikely that both the polar nuclei should come from the same pole of the embryo-sac.

Embryo-sacs with less than 8 nuclei.—Monosporic embryo-sacs with less than 8 nuclei are known to arise sometimes as a result of the suppression of some divisions in the chalazal part of the embryo-sac (see Pardi, 1937, on *Serapias lingua*).²

A remarkable anomaly has, however, been reported by Billings (1937) in the development of the embryo-sac in *Isomeris arborea*. There is a single hypodermal archesporial cell which cuts off about four wall cells. “After their production megaspore formation does not generally follow, but when it does occur the non-functional megaspores soon disintegrate”. The nucleus of the “embryo-sac mother cell” divides to form the 2-nucleate stage and thereafter the primary chalazal nucleus alone divides once giving rise to 3 nuclei in the mature embryo-sac. Two form the synergids and the remaining is an “endosperm” (= polar) nucleus. No egg cell is differentiated; fertilisation does not occur since the pollen tube does not discharge its contents into the embryo-sac and the embryo arises from a group of endosperm nuclei.

Several of the above statements appear to be of a doubtful nature. To mention a few instances, Fig. 19 which would ordinarily be interpreted as a clear tetrad of megaspores of which the lowest is dividing, is regarded by him as showing 3 wall cells and an embryo-sac mother cell. Similarly Fig. 18 in which the axial row clearly consists of one wall cell and 4 megaspores, of which the lowest has enlarged, is regarded as composed of 4 parietal cells and “a vacuolated sporogenous cell”. Various other ingenious interpretations have been offered which cannot be discussed here in detail. It is sufficient to state that the illustrations entirely fail to support the conclusions based upon them.

² Treub (1911) reported a 5-nucleate embryo-sac in *Garcinia* and Rutgers (1923) in *Moringa*. Both of these have been shown to be incorrect (Puri, 1939, 1941). The embryo-sacs are absolutely normal, but the antipodals are frequently very ephemeral.

Embryo-sacs with more than 8 nuclei.—This condition may be frequently encountered as a result of some secondary increase in the number of antipodals. Abnormalities in the other constituents of the embryo-sac are very rare. A few examples are cited below.

In *Crepis capillaris* (Gerassimova, 1933) and *Taraxacum kok-saghyz* (Poddubnaja-Arnoldi and Dianowa, 1934) occasionally 2-4 egg cells may be present besides the other elements of the embryo-sac. The origin of the supernumerary eggs was not clear; gradually they all degenerate.

In *Pyrethrum cinerariaefolium* (Martinoli, 1939) the female gametophyte may occasionally have 9 nuclei instead of 8. According to the author's statement this is due to a division of the egg, the additional cell passing to the centre of the embryo-sac. Sometimes even 10 nuclei may be present, of which 3 lie at the micropylar end, 2 in the centre (one is the additional cell derived from the egg and the other is the nucleus formed by polar fusion), and 5 at the chalazal end.

*Embryo-sac haustoria.*³—Haustorial structures may be formed from the embryo-sac itself or from some of its constituent parts. In several members of the Amaranthaceæ the chalazal part of the embryo-sac grows actively and penetrates further into the nucellus while the antipodals are left *in situ* and thus become laterally placed (Kajale, 1940). In *Suriana maritima* (Anantaswamy Rau, 1940) the lower end of the embryo-sac grows considerably during the post-fertilisation stages and forms a long tubular structure piercing into the chalaza.

In other cases it is the micropylar end that elongates. In *Putoria* (Eagerlind, 1936) and *Kirengeshoma* (Mauritzon, 1939) the embryo-sac grows a considerable way upward into the micropylar canal. In *Arechavaletaia uruguayensis*, Ventura (1937) reports that the embryo-sac protrudes out of the endostome and comes to lie in the exostome formed by the outer integument. On the other hand Karsten's (1891) statement that in *Sonneratia* the embryo-sac pierces through the tip of the nucellus is shown to be incorrect by Mauritzon (1939). This tubular structure is really the broad pollen tube entering into the nucellus.

The Lorantheæ present the strangest behaviour in this respect. *Dendrophthoe falcata* has recently received a detailed investigation at the hands of Singh (unpublished). The megaspore mother cells give rise to rows of 4-8 megaspores. Many of these begin to elongate immediately (*cf.* Eagerlind, 1937, on the Rubiaceæ) and this elongation is so pronounced in later stages that the mature embryo-sacs wind their way upward into the style; the lower end is not able to make much progress being checked by a collenchymatous pad at the base of the ovary.

³ A fuller account of the different types of haustorial structures met with in the ovule, both before and after fertilisation, will be published in a subsequent paper on the "Nutrition of the embryo-sac in Angiosperms".

Haustorial synergids with elongated tips are frequently met with in the long and narrow embryo-sacs of some of the Sympetalaë. In *Allium* (Weber, 1929; Sundar Rao, 1940), *Berberis* and *Mahonia* (Mauritzon, 1936) and some other plants, one of the synergids becomes enlarged and persists for some time during the development of the embryo. In *Isomeris arborea*, according to Billings (1937), the embryo-sac has no egg and occasionally one of the synergids enlarges and undergoes a series of divisions giving rise to a fairly long chain of cells. In one case a bulbous synergid containing free nuclei has been figured.

The antipodal tissue frequently serves a haustorial function by an increase in the size or the number of its cells. In some members of the Rubiaceæ (Fagerlind, 1937) the lowest antipodal undergoes a considerable elongation towards the chalaza and serves to draw nutrition.

A discussion of the various types of haustoria formed by the endosperm is beyond the scope of this paper since such developments take place considerably after fertilisation. Mention may, however, be made of the work of Srinivasan (1940) on *Angelonia* in which he says that endosperm haustoria are absent and instead it is the synergids which are persistent and haustorial. *Angelonia* is being reinvestigated by C. V. K. Iyengar and it remains to be seen whether Srinivasan's account receives any confirmation. See also Maheshwari and Navalakha (1941).

OENOTHERA-TYPE

The embryo-sac is monosporic but it generally arises from the micropylar megaspore of the tetrad. Occasionally the chalazal may function, however, and sometimes both continue to develop for a time (twin embryo-sacs), although eventually one is usually suppressed. The most characteristic feature of the *Oenothera-type* is that immediately after tetrad formation the nucleus of the functioning megaspore moves to the upper portion of the cell where it divides twice to give rise to 4 nuclei which form the 2 synergids, one egg and a single polar nucleus. There is no third division and consequently the chalazal quartet is absent.

This type is restricted to the family Oenotheraceæ, all of whose plants that have so far been investigated closely correspond to it. The only exception was *Trapa* (Ishikawa, 1918) which has now been removed to a separate family, Hydrocaryaceæ (see Engler, 1936). It may, however, be noted that even in *Trapa* the antipodals are very ephemeral and the closely allied family Lythraceæ already shows a tendency towards reduction in the chalazal part of the embryo-sac (see Joshi and Venkateswarlu, 1936, and the literature cited by them). Not only are the antipodals ephemeral here but sometimes there is a suppression of the divisions in the chalazal end of the embryo-sac, leading to a 6-nucleate condition instead of the usual 8-nucleate one. It seems clear that the *Oenothera-type* of embryo-sac, in which the

chalazal quartet is completely gone, has been derived as the result of a continuation of this tendency.

Among the latest embryological works on the family is that of Beth (1938). Although concerned mainly with the problem of adventive embryony as brought about by wounding, he also makes some interesting observations on the embryo-sac. Occasionally he saw twin embryo-sacs in *Oenothera lamarckiana* arising as the result of a concurrent development of the 2 megaspores of a tetrad, and once he encountered a "Drillingsembryosack" formed by the development of 3 megaspores. It could not be ascertained, however, whether all the three megaspores came from the same tetrad, or different ones, although the former condition is held more probable.

Beth (1938, p. 323) also came across two embryo-sacs showing an inversed orientation, with the egg apparatus situated towards the chalazal end and the polar nucleus towards the micropylar. Both were observed in ovaries of *O. lamarckiana* and *O. gigas* that had been punctured for the induction of adventive embryony, but he thinks it is very improbable that this had anything to do with the reversed polarity. Previously Täckholm (1915) had observed two similar cases in *Fuchsia* "*Marinka*" and *F. procumbens*. Beth thinks that such a reversion of the normal polarity is probably caused by cold or by a disturbance in the nutritional physiology of the plant.

Khan (1941) has studied the development of the embryo-sac in *Jussieua repens*. In one case he observed a 3-nucleate embryo-sac with a single synergid, probably formed by the primary synergid nucleus having failed to divide. In another case, the nucleus of one synergid had divided into two and that of the other was in the process of division by budding.

ALLIUM-TYPE

Since my last review was written the *Allium-type* has been demonstrated in a number of additional cases and some of the older records have been proved false. All such cases are cited here under their respective families.

Podostomaceæ.—In *Podostemon subulatus* according to Magnus (1913), the megaspore mother cell divides to form two dyad cells, of which the lower undergoes two divisions to form a 4-nucleate embryo-sac having an egg, two synergids and a single polar nucleus. This account has been the basis of the so-called "*Podostemon-type*" of embryo-sac. Hammond (1937) has recently investigated another species of the same genus *P. ceratophyllum*, in which the development is clearly of the *Allium-type*, although there is considerable reduction in the chalazal end. After the 2-nucleate stage the primary chalazal nucleus remains undivided and soon degenerates and disappears; the micropylar produces the two synergids, an egg and a single polar nucleus.

Hammond's account corresponds closely with that of Went (for full citations see Maheshwari, 1937) for several other members of the family and it now seems very doubtful whether the *Podostemon* and *Dierca*-types of Magnus (1913) exist at all.

Loranthaceæ.—Following his previous account of the embryology of *Korthalsella dacrydii* (1935) Rutishauser has now published another paper dealing with 2 other plants of the same family, *Korthalsella opuntia* and *Ginalloa linearis* (1937). In all the 3 cases the development is of the *Allium*-type. The central papilla has 2 archesporial cells. Each produces a dyad of which the upper cell is larger and functions, and the lower soon degenerates. After the 4-nucleate stage there is a slow but steady curvature in the embryo-sac, which causes its lower part to bend out of the papilla and proceed upward into the carpellary tissue. Meanwhile the four nuclei divide to form 8. The egg apparatus differentiates in the originally lower portion of the sac which now occupies a higher position.⁴

Miss Stevenson (1934) investigated the morphology of two spp. of *Korthalsella*, *K. Lindsayi* and *K. salicornioides*. According to her the megaspore mother cells divide to form linear tetrads of megaspores of which the upper or lower cell may develop into an embryo-sac. Since Stevenson's paper is mainly morphological and only a few stages of the embryo-sac are figured, her conclusions on this point must receive confirmation.

Balanophoraceæ.—Umiker (1920) thought that *Helosis guyanensis* Reih. (= *H. cayennensis* Schwarz) is an apomict and described the development as of the *Adoxa*-type (without any reduction in chromosome number), but this now seems to be incorrect. Fagerlind (1938c) has shown that reduction and fertilisation occur normally. The development of the embryo-sac is of the *Allium*-type. After the 8 nuclei have been formed, the micropylar quartet gives rise to an egg apparatus and the upper polar nucleus. Of the 4 nuclei at the chalazal end, as a rule, two move up and fuse with the upper polar nucleus and the remaining two organise into a single binucleate or two uninucleate antipodal cells. Sometimes all the 3 antipodal nuclei are present in their proper positions, in which case there are only two polar nuclei.

Another genus *Ditepalanthus* recently investigated by Fagerlind (1938b) seems to be similar to *Helosis*.

⁴ In my previous paper (1937, p. 366, footnote 2) I had stated that the embryo-sacs of the *Loranthaceæ*, *Balanophoraceæ* and some *Gentianaceæ* (Oehler, 1927) afford the best instances of inverted polarity. I now think that the inversion is more apparent than real. It may well be that the ovules are actually anatropous but due to the lack of any integuments or micropyle it is impossible to apply the usual criteria for judging this. Further comparative studies are necessary to clear up this point (see also Joshi, 1939a).

Euphorbiaceae.—D'amato (1939), who has investigated the embryology of several spp. of *Euphorbia*, writes that in most spp. the embryo-sac is normal 8-nucleate, but some spp. show the *Scilla*-type (= *Allium*-type) either occasionally or regularly.

Malpighiaceae.—Stenar (1937) reported an *Allium*-type of embryo-sac in *Galphimia gracilis* and Subba Rao (1939) in *Malpighia glauca*. Other spp. of the genus *Malpighia* as well as most other plants of this family have tetrasporic embryo-sacs of the *Penaea*-type.

Oleaceae.—Andersson (1931) had already reported an *Allium*-type of embryo-sac in *Olea chrysophylla* and *O. europaea*. King (1938) has confirmed it in the latter species.

Convolvulaceae.—Johri and Nand (1934) reported an *Allium*-type of embryo-sac in *Cuscuta reflexa* with the lower dyad functioning. Other spp. of this genus are, however, quite normal (see Fedortschuk, 1931; Smith, 1934; Finn, 1937). Such a difference is possible but not so probable and it seems that Johri and Nand's observations need confirmation.

Polemoniaceae.—Jönsson (1879-80) studied the development of the embryo-sac in *Polemonium coeruleum* and reported an *Allium*-type in this plant. This was considered as doubtful in my last paper (1937, p. 377). This scepticism was well justified as Sundar Rao (1940b) has recently figured a clear linear tetrad of megaspores in this plant and thus shown the development to correspond to the *Normal*-type.

Solanaceae.—Several species of the genus *Nicotiana* and their hybrids have been investigated during recent years by Modilewski (1936, 1937a, b) and Modilewski and Dzubenko (1937). In some of them the development is of the *Normal*-type and the embryo-sacs are monosporic; in others the development is stated to be of the *Allium*-type and the embryo-sacs generally arise from the lower dyad cell.

Butomaceae.—Johri (1938a and b) has now published the results of his studies on *Limnocharis emarginata* and *Hydrocleis nymphoides*. With regard to the former plant, the interpretations of Hall (1902) and Nitzschke (1914) are criticised. According to the former author the embryo-sac is tetrasporic and 5-nucleate, while according to the latter it is monosporic and usually six but sometimes 7- or 8-nucleate. Johri's observations prove that it is really bisporic, arising from the lower dyad cell formed after the first division of the megaspore mother cell. Generally the mature embryo-sac is 5-nucleate. *Hydrocleis nymphoides* is similar to the above. Suessenguth's (1921) account is incorrect.

Gramineae.—Stover (1937) quotes Schnarf (1926) as reporting *Scilla*-type in *Coleanthus subtilis*. This is incorrect as Schnarf himself figures a clear T-shaped tetrad in this plant.

Commelinaceæ.—Walker (1938b) has recently described the development of the embryo-sac in *Tradescantia paludosa* under normal conditions and after treatment with colchicine. In the first case the embryo-sac develops from the chalazal dyad cell and is 8-nucleate. In the latter case there is a disturbed condition; spindle fibres are not formed and the arrangement of the nuclei becomes irregular.

Liliaceæ.—Jones and Emsweller (1936) find that in *Allium cepa* the chalazal dyad cell undergoes three divisions to form an 8-nucleate embryo-sac. One of the synergids becomes hypertrophied and probably serves as a nutritive organ. *Allium gorganianum* (Sundar Rao, 1940) is similar. One of the antipodal cells becomes egg-like.

A peculiar course of development has been reported in *Allium mutabile* (Porter, 1936). The megaspore mother cell divides to form two dyad cells of which the micropylar promptly begins to degenerate. The other is said to divide and form 2 megaspores of which the chalazal functions. The primary chalazal nucleus (of the 2-nucleate stage) is very small and the same difference is noticeable at the 4-nucleate stage between the two micropylar and the two chalazal nuclei. Now one of the micropylar nuclei divides to form the egg and a synergid, and the other divides to form the two polar nuclei; the two nuclei at the chalazal end are said to degenerate and disappear!

If Porter's statements are correct, the development should be regarded as of the normal monosporic type. It seems, however, that a reinvestigation is necessary. The following points deserve a critical study:—

(i) How has the row of 3 cells shown in Fig. 6 originated? Neither this figure nor any other gives any definite evidence in favour of the author's view that the lower dyad cell divides into 2 cells. It is just as likely that the micropylar dyad cell has divided in which case the embryo-sac would be bisporic.

(ii) The statement that the egg and synergid are sister cells as also the polar nuclei is also unsupported by the figures. The requisite stages between Figs. 9 and 10 are missing and one may be justified in supposing that the embryo-sac in Fig. 10 shows only 4 nuclei not due to any real reduction but because of the early degeneration of the antipodals and one synergid.

In *Leucocoryne ixiioides* (Cave, 1939), "the macrospore mother cell divides giving rise to two macrospores⁵ one of which degenerates. The other divides 3 times giving rise to 8 nuclei which become arranged in the normal way."

Ruscus aculeatus (De Philippis, 1936) is subdioecious, some flowers are definitely carpellate, others when in bud, show both carpels and stamens, but later the carpels degenerate leaving the

⁵ These are really the dyad cells.

flower staminate. A very few flowers continue to ripen with both stamens and carpels. Development of the female gametophyte in the carpellate flowers is said to be of the *Scilla-type* (bisporic) while in flowers with stamens it is said to be of *Normal-type* (monosporic).

In *Iphigenia indica* (Joshi, 1939) the embryo-sac occasionally develops according to the *Allium-type*. One case is figured in which no wall is formed in the chalazal dyad cell after the second meiotic division, but the nucleus formed towards the micropylar side is small and likely to degenerate during further development. The embryo-sac in this case would develop from the nucleus of only one megaspore but the cytoplasm of two. It is thus considered to be intermediate between the *Normal-* and *Allium-types*.

Jeffrey and Haertl (1939) state having studied several species of *Trillium* (*T. grandiflorum*, *T. erectum*, *T. undulatum* and *T. sessile*), the material having been collected from wild as well as cultivated plants. "The reduction division in the embryo-sac mother cell is quite normal in contrast to the situation in the pollen mother cells, and 5 chromosomes are present. One of the derivatives of the mother cell survives, as is usually the case, and gives rise to an embryo-sac. Ordinarily this contains only 4 nuclei, a situation paralleled by the Onagraceae and allied forms, as well as by certain Orchids. Of the four nuclei one becomes the egg and the other an abortive synergid. The remaining two nuclei fuse together and form the endosperm nucleus." There is no fertilisation; the egg develops into a feeble haploid embryo, which ultimately aborts. The endosperm, which is diploid, forms in the usual manner and first contains a large central cavity around which are rapidly dividing cells. "After the abortion of the haploid embryo and after the endosperm has reached a considerable degree of development, a diploid embryo makes its appearance in the micropylar region of the endosperm and is continuous with the endosperm tissues. This is the embryo which perpetuates the plant."

The whole development is so abnormal that it seems proper to reserve comment till the full paper is available.

Orchidaceae.—Stenar (1937) reports that in *Achroanthes monophyllas* the chalazal dyad cell functions. At the 4-nucleate stage there is an appreciable difference in the relative sizes of the micropylar and the chalazal nuclei. The latter are much smaller and usually do not divide further, so that the mature embryo-sac is 6-nucleate.

PEPEROMIA-TYPE

Penaea form :—

During the last four years several new cases of this type have been reported in the families Malpighiaceae, Combretaceae and Plumbaginaceae.

Malpighiaceae.—In all the plants mentioned below the organisation of the embryo-sac is quite typical :—

1. *Hiptage madablota* .. Subba Rao, 1937, 1940.
2. *Banisteria laurifolia* .. Subba Rao, 1937, 1940.
3. *Stigmatophyllum aristatum* .. Subba Rao, 1937, 1940.
4. *Malpighia urens* .. Stenar, 1937.
5. *M. coccifera* .. Subba Rao, 1939.
6. *M. puniceifolia* .. Narasimhachar, 1938.
7. *Tristellitia australis* .. Subba Rao, 1939.

Combretaceæ.—In *Combretum pincianum* and *C. paniculatum* (Mauritzon, 1939) four quartets are organised of which the micropylar and the chalazal give rise to 2 egg-apparatuses, composed of an egg cell and 2 synergids. Their polar nuclei and 4 other free nuclei from the lateral quartets migrate towards the centre and a varying number of these come together to form one or two fusion nuclei. The remaining 4 nuclei surround themselves with cell-membranes and lie irregularly on the lateral sides of the embryo-sac. Occasionally their walls become disintegrated and the nuclei become free thus adding to the number of polar nuclei already present.

Plumbaginaceæ.—*Statice-Eu-Limonium*, formerly understood to have an *Adoxa*-type of embryo-sac (Dahlgren, 1916), has been reinvestigated by Eagerlind (1938d) and found to belong to the *Penaea*-type. The 16 nuclei are arranged in four groups, of which the lateral degenerate quickly.

Drusa-form:—

Eagerlind (1937) has recently published an important monograph on the embryology and cytology of the Rubiaceæ. Several spp. of the genus *Crucianella* (*C. latifolia*, *C. græca*, *C. angustifolia*, *C. imbricata* and *C. ægyptiaca*) were investigated and the results were found to differ in some important particulars from those of Lloyd (1902). The number of megaspore mother cells in a nucellus is very large (about 20) and several of them undergo reduction divisions simultaneously. The four megaspore nuclei are unseparated by walls and lie in a single row with vacuoles intervening between them. Each nucleus divides twice in succession, resulting in a total of 16 nuclei of which 4 lie in the somewhat swollen micropylar part and 12 in the chalazal. The former give rise to the egg apparatus and the upper polar nucleus. Of the latter, one nucleus functions as the lower polar and the remaining as antipodals. Some of the antipodal cells are 2-nucleate so that their total number may be less than 11. A more or less similar type of development was seen in *Rubia olivieri* and is inferred in *Diodia virginiana* studied by Lloyd (1902).

In *C. latifolia* only 15 nuclei are seen. This is because one of the chalazal nuclei of the 8-nucleate stage fails to divide. The number of antipodal nuclei is, therefore, only 10.

In *Ulmus* spp. (see p. 248) also there is a probability of the occurrence of this type of embryo-sac.

Campbell's account of the embryo-sac of *Pandanus* has never been very clear, but judging from what he states in his book entitled

"Evolution of Land Plants" (1940, p. 553) it seems that the development is a modification of the *Drusa-type*. He writes as follows: "In *Pandanus* the first stages follow the usual course and there are 2 micropylar and 2 antipodal nuclei; the 2 micropylar nuclei divide once and there is the formation of a typical egg apparatus and a polar nucleus. The antipodal nuclei divide repeatedly, forming usually 12 large, free nuclei. By further divisions there may be formed more than 60 nuclei, which in the later divisions are separated by cell walls and form a mass of prothallial tissue. Some of the nuclei become free and assume the rôle of polar nuclei, fusing with the upper polar nucleus into a large endosperm nucleus."^{6a}

Acalypha indica-form :—

Some material of *Acalypha indica*, collected from Agra and investigated by Maheshwari and Johri⁶ (1940) showed a tetrasporic 16-nucleate embryo-sac with the nuclei arranged at first in 4 quartets as in the Penæaceæ. The organisation of the mature embryo-sac presented a great variation, however. In most cases the cytoplasm aggregates around 2 nuclei of each quartet and they become separated towards the periphery by the formation of a limiting membrane. Two nuclei of each quartet, which have remained free, migrate to the centre so that the secondary nucleus formed by their fusion is octoploid.

In a few cases the organisation was like that of the Penæaceæ with 4 groups of 3 cells each and only 4 nuclei in the centre (Stephens, 1909). Occasionally there was a lack of any definiteness in organisation.

This type of embryo-sac seems to be most closely related to the *Penæa-type* on the one hand and the *Plumbago-type* on the other.

FRITILLARIA-TYPE

The *Fritillaria-type* was so fully discussed in my last paper that there is not much to be added to what I wrote at that time. Additional plants, in which it has been recently demonstrated, are:—*Lilium philippinense* (Santos, 1937), *Lilium tigrinum* (Westfall, 1940), *Gagea fascicularis* (Joshi, 1940), *Tulipa gesneriana* (Simoni, 1937), *Armeria bupleuroides* (Fagerlind, 1938) and *Piper longum* (Joshi, unpublished).

Special mention must be made of the Tamaricaceæ.

Myricaria germanica, originally investigated by Frisendahl (1912) and reported to have an *Adoxa-type* of embryo-sac has been

⁶ The full paper is in the press since a long time, but publication has been delayed due to the war.

^{6a} Fagerlind (1940) has reinvestigated *Pandanus* and reports that the embryo-sac is monosporic and normal but some nuclei of the adjacent cells at the chalazal end get included and divide independently, thus resulting in a many-nucleate condition.

the subject of much discussion, since the publication of Bambacioni's work on *Fritillaria* (1928). Zabban (1936) has settled the matter by demonstrating the 1+3 arrangement of the nuclei at the primary 4-nucleate stage and a fusion of the 3 spindles in the chalazal end of the embryo-sac.

Furthermore, Puri (1940) reports the same type of embryo-sac in *Tamarix chinensis* and Sharma (1939, 1940) in *T. ericoides*. On the other hand, Mauritzon (1936) found the *Adoxa*-type in the spp. of this genus investigated by him.

In *Tamarix gallica* Pàroli (1937, 1939) has shown that the development may proceed in two ways, either according to the *Fritillaria*-type or according to the *Adoxa*-type. This seems to explain the divergent results obtained by previous investigators on this genus.

Joshi (1938) reviewed the occurrence of the *Fritillaria*-type of embryo-sac and interpreted its occurrence in widely separated families as an argument against the monophyletic origin of Angiosperms. For a discussion and criticism of this view see Maheshwari (1939).

PLUMBAGELLA-TYPE

As stated under the *Plumbago*-type, Dahlgren (1915, 1916) was the first to investigate the embryo-sac of *Plumbagella micrantha* and his account remained unchallenged until very recently. Schnarf (1936) expressed a note of caution in accepting these results and stressed the need for a reinvestigation. Dahlgren (1937), in his paper on *Plumbago*, withdrew his previous observations on this genus and *Ceratostigma* but still considered his work on *Plumbagella* to be so thorough as to preclude any probability of its embryo-sac being similar to that of *Plumbago*. He admits, however, the advisability of a reinvestigation and this has been done almost simultaneously by Fagerlind (1938a) and Boyes (1939a and b).

As a result of these works it has been discovered that the old *Plumbagella*-type, with the four megaspore nuclei directly taking part in the organisation of the embryo-sac, becomes untenable. Instead, a new type of embryo-sac has been discovered which shows certain resemblances with the *Fritillaria*-type, but is yet distinctive enough to be retained as a separate unit. The name "*Plumbagella*-type" must, therefore, remain although in a different sense altogether.

Briefly speaking, the development is as follows: The nucleus of the megaspore mother cell undergoes the 2 reduction divisions in the normal way. Soon after the 4 nuclei are formed, the micropylar surrounds itself with a zone of dense cytoplasm and begins to enlarge. The other three nuclei which lie closer to the chalazal end, migrate still further downwards and become separated from the micropylar by a large vacuole. Here they fuse together to

form a single triploid nucleus.⁷ This fusion may not proceed simultaneously; frequently two of the nuclei fuse first and the third follows afterwards. This is the secondary 2-nucleate stage in which the micropylar nucleus is obviously haploid and the chalazal triploid—a fact that was also confirmed by chromosome counts (Boyes, 1939b, p. 542). Very soon both of these nuclei divide to form 2 pairs (secondary four-nucleate stage), the two micropylar nuclei being haploid ($n = 6$) and the chalazal triploid ($n = 18$). Generally there is no further division. Now the nucleus nearest the micropyle organises itself into the egg, the triploid nucleus nearest the chalazal end becomes cut off with some cytoplasm to form a single antipodal cell and the remaining two nuclei, the upper of which is haploid and the lower triploid, fuse to form a tetraploid secondary nucleus.⁸ If double fertilisation takes place normally, the primary endosperm nucleus and its derivatives should be pentaploid.

The essential difference between the type of development as described here and that in *Fritillaria* is that in the latter case there is a fourth nuclear division following the secondary 4-nucleate stage. Another difference, which is, however, of lesser importance, is that in *Plumbagella* the fusion of the 3 chalazal nuclei takes place more rapidly than in *Fritillaria*. In the former they unite before entering the prophases of the third nuclear division; in the latter they form separate spindles which later become united into a common mass.

PLUMBAGO-TYPE

The first work of importance on the Plumbaginaceæ is that of Dahlgren (1915) who investigated 16 plants of this family belonging to 8 different genera:—

Staticeæ.—

1. *Acantholimon glumaceum* Boiss.
2. *Armeria alpina* Willd.; *A. plantaginea* Willd.; *A. vulgaris* Willd.
3. *Goniolimon collinum* Boiss.; *G. tataricum* (L.) Boiss.
4. *Limoniastrum monopetalum* (L.) Boiss.
5. *Statice bahusiensis* Fr.; *S. gmelini* Willd.; *S. macroptera* Webb. and Berth.; *S. sinuata* L.

⁷ It must be said on behalf of Dr. Dahlgren that such fusions were entirely unknown until Bambacioni (1928) first discovered them in *Fritillaria*, and it is no wonder, therefore, that he was led astray and made some wrong interpretations. In recent embryological literature Bambacioni's work must rank as of a very high order. It has led to the rectification of scores of errors and added much to our knowledge in other ways.

⁸ Approximately 4–5% of the embryo-sacs were found to be 6-nucleate (Boyes, 1939b). Their origin is traced back to an irregularity at the time of migration of the 3 megaspore nuclei to the chalazal end. At this stage it sometimes happens that only two nuclei move to the chalazal end and become separated from the third by a vacuole this latter nucleus being itself separated by a vacuole from the micropylar. Apparently the 2 nuclei which reach the chalazal end fuse together to form a single diploid nucleus and all the three nuclei now divide once to form six—2 lying at the micropylar end (haploid), 2 lying in the central region of the embryo-sac (also haploid) and 2 at the chalazal end (diploid).

Plumbagineæ :—

6. *Ceratostigma plumbaginoides* Bunge.
7. *Plumbago capensis* Thunb., *P. zeylanica* L., *P. pulchella* Boiss.
8. *Plumbagella micrantha* (Ledeb.) Spach.

Of these the first five genera, belonging to the *Staticeæ*, were reported to have an 8-nucleate embryo-sac of the *Adoxa*-type, while the last three, of which *Plumbagella micrantha* was investigated in the greatest detail, were reported to have a 4-nucleate embryo-sac of an entirely different type. According to his statement the megaspore mother cell, in these cases, divides twice without wall formation to form the four reduction nuclei, equivalent to the

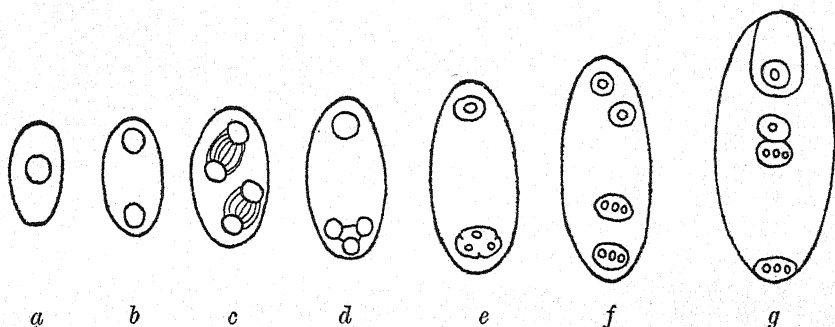


Fig. 2.—Diagram illustrating the development of the embryo-sac in *Plumbagella micrantha*. Stages *d*, *e*, *f* were missed by Dahlgren, leading him to an incorrect interpretation. For further explanation see text.

megaspores. The micropylar pair gives rise to the egg and upper polar nucleus and the chalazal pair to a single antipodal and lower polar nucleus. This was considered to be the most reduced type of embryo-sac yet discovered and the situation was compared with that in animals during the maturation of the egg.

Dahlgren's observations aroused much interest and were warmly received by other embryologists. A reinvestigation of *Plumbago capensis* made twenty years later (Haupt, 1934) revealed, however, that the four nuclei divide once again and the embryo-sac is thus really 8-nucleate. Dahlgren's⁹ (1937) own reinvestigation of *P. zeylanica* has entirely confirmed the observations of Haupt; Fagerlind¹⁰ (1938a) has shown the same thing in *Ceratostigma* and

⁹ The confession of a mistake on the part of so sincere a worker as Dr. Dahlgren may be quoted in his own words (see Dahlgren, 1937, p. 488) "Begeistert wie ich damals war wegen der sensationellen Entwicklung bei *Plumbagella micrantha* zweifelte ich keinen Augenblick daran, dass der Embryosack auch bei den anderen Plumbaginoideen in derselben Weise entstehe wie bei dieser Art."

¹⁰ Fagerlind has also confirmed the observations of Haupt (1934) and Dahlgren (1937) on *Plumbago capensis* and *P. zeylanica* respectively.

Mathur (1940) in the hitherto uninvestigated *Vogelia indica*. Boyes (1939b) adds three more plants to the list:—*Plumbago scandens*, *P. coccinea* and *Ceratostigma willmottianum*. In all these cases the commonest condition is that of the 8 nuclei, three quickly degenerate, four fuse in the centre to form the secondary nucleus and the single nucleus remaining at the micropylar end is cut off by a wall to form the egg. The fusion of the polar nuclei may proceed by steps so that occasionally some of them may be seen to have already fused while others are still free.

It is now possible to understand the "aberrant" cases found by Dahlgren (1915) in *Ceratostigma* and *Plumbago*, which he was then unable to interpret in a satisfactory manner (see Haupt, 1934, p. 657). Occasionally more than 4 nuclei were seen in embryo-sacs that had not yet been fertilised and once in *Ceratostigma* he saw an embryo-sac with all the 8 nuclei. Twice in *Ceratostigma* and once in *Plumbago zeylanica* he encountered embryo-sacs containing four free nuclei (now interpreted as polar nuclei) in addition to the egg. All these were unfortunately dismissed at that time as accidental variations and their true significance remained obscure until recently. The mature embryo-sac is 2-nucleate (one egg and a secondary nucleus) according to both Dahlgren and Haupt.

As regards the derivation of the *Plumbago*-type of embryo-sac, it seems that it is closely related to the types seen in the Penæaceæ investigated by Miss Stephens (1909) (see Haupt, 1934, and Fagerlind, 1938) and *Acalypha indica* (Maheshwari and Johri, 1940). Of the 8 nuclei in the embryo-sac of *Plumbago*, the 4 peripheral ones separated by walls may be considered as potential "eggs," the remaining four being regarded as polar nuclei. This is supported by the fact that both Dahlgren (1916, 1937) and Haupt (1934) occasionally observed the peripheral cells to grow large and become egg-like and recently Mathur and Khan (1941) have reported the same thing in *Vogelia indica*. Unfortunately, none of these workers has yet attempted a detailed investigation of the embryogeny and it still remains to be seen whether the remaining 3 "eggs" can also occasionally develop into embryos. It must, of course, be admitted that the micropylar egg, being more favourably placed than others for fertilisation, is the one that will develop in the majority of cases.

Transitions from tetrasporic 16-nucleate to tetrasporic 8-nucleate embryo-sacs are not unknown (see Palm, 1915, on *Tanacetum*; Westergård, 1936, on *Gagea*; and Fagerlind, 1937, on *Crucianella*). In *Plumbago* itself Fagerlind (1938a, p. 7) occasionally saw as many as 14 nuclei and Dahlgren (1916) mentions a similar thing in *Armeria*.

ADOXA-TYPE

The *Adoxa*-type, previously called "*Lilium*-type" and supposed to have a fairly wide distribution, especially among the monocots, is really of very rare occurrence. The appearance of the mature embryo-sac is entirely like that of the normal 8-nucleate type, but

the origin is quite different. In the *Adoxa-type*, the 4 megaspore nuclei, which are not separated by walls, divide only once to form the 8 nuclei of the mature embryo-sac, while in the *Normal-type* a single megaspore takes part in the development and three divisions take place before the stage of differentiation of the egg is reached. These relations are clearly brought out in Fig. 1.

At present there are only a few plants in which an embryo-sac of this type is known to occur:—

(1) *Adoxa moschatellina*.—This plant was first investigated by Jönsson (1879-80), whose account was confirmed in 1909 by Lagerberg. A more detailed investigation has been made very recently by Fagerlind (1938d), who also had at his disposal the preparations of Lagerberg. The embryo-sac is clearly tetrasporic and vacuolation begins only after the 4 megaspore nuclei have been formed. All the 4 nuclei divide once. Of these the two lying nearest the micropylar end and as well as the two lying nearest the chalazal end are very small, while the remaining four are almost equally large and conspicuous. The mature embryo-sac has 2 small and ephemeral synergids, 3 antipodal cells of which two are very small, an egg cell and 2 polar nuclei.

(2) *Sambucus racemosa*.—Jönsson (1879-80), who first investigated this plant, reported a *Normal-type* of development. Lagerberg (1909) found an *Adoxa-type*. Fagerlind (1938) re-examined the original preparations of Lagerberg and confirms that the embryo-sac is tetrasporic in this species as well as *S. ebulus*, newly investigated by himself. He did not, however, have sufficient material at his disposal to decide whether the development is of the *Adoxa-* or *Fritillaria-type*. Further work is, therefore, necessary to clear up this point.

(3) *Tulipa ostrovskiana* (Romanov, 1938).—The four megaspore nuclei are arranged in 2 pairs, one at each pole of the embryo-sac. All of them divide once to give rise to 8 nuclei which organise in the usual way, to form a normal-looking embryo-sac.

(4) *T. tetraphylla* (Romanov, 1938).—After meiosis, 3 of the megaspore nuclei move to the upper part of the embryo-sac and one to the lower. Then all divide once, forming 6 nuclei in the upper end and 2 in the lower. The micropylar part of the embryo-sac now organises 5 cells (one of which becomes the egg) and the chalazal organises one cell (the antipodal, which soon dies); the 2 free nuclei left in the middle of the sac are the polars.

(5) *Limnanthes Douglasii* (Eysel, 1937).—A reinvestigation of this plant has confirmed Stenar's report that the embryo-sac is of the *Adoxa-type*. Differences in the sizes of the nuclei are quite frequent, those towards the chalazal end being progressively smaller in many cases. The antipodals are thus very small from the beginning and quite ephemeral in most cases. The synergids on the other hand, are large and prominent and undoubtedly play an important rôle in the nutritional physiology of the embryo-sac.

(6) *Erythronium albidum* (Cooper, 1939).—Of the four megaspore nuclei, the two micropylar and the upper chalazal divide normally. The remaining lowest nucleus enters upon a more or less abortive division. The organisation of the mature embryo-sac is thus similar to that of *Lilium* but the origin is different.

In my last article I gave a list of the cases in which the *Adoxa-type* had been reported but which were shown or suspected, on good reasons, to be incorrect. Since then some other cases of a similar nature have been brought to light:—

In the *Ulmaceæ*, a tetrasporic 8-nucleate embryo-sac of the *Adoxa-type* is known to occur in *Ulmus americana* (Shattuck, 1905), *U. hollandica belgica*, *U. wilsoniana* and *U. pumila pinnatoramosa* (Leliveld, 1935) and *U. fulva* (Walker, 1938 a). Shattuck (1905) mentions, however, that occasionally the embryo-sac may show 8–16 or even more free nuclei, but this condition was not fully investigated. Leliveld's work is even more brief, but she states that occasionally there may be more than 3 antipodals, although always in a rudimentary state. Walker (1938a) once saw a 12-nucleate embryo-sac in *U. fulva*. Fagerlind (1938d) saw a preparation of an unidentified species of *Ulmus* in Prof. Rosenberg's collection in which an embryo-sac which was still young showed 2 nuclei at the micropylar end and six at the chalazal. Some fixed material of *Ulmus* (specific name not mentioned), also provided by Prof. Rosenberg and studied by Fagerlind, showed mature embryo-sacs in which the antipodal cells were always found to be more than 3, although their number could not be exactly counted because degeneration had already set in. From a critical study of the figures and statements of other authors as well as of his own preparations, Fagerlind suspects that the embryo-sac of *Ulmus* is probably 16-nucleate and the development corresponds with the *Drusa-form* described by Håkansson (1923, 1927). Occasionally the number of nuclei may be less than 16 due to failure of the last division in some of the chalazal nuclei.

In the *Santalaceæ* two spp. of the genus *Thesium*, *T. intermedium* and *T. montanum*, investigated by Modilewski (1928) and Schulle (1933) respectively, are reported to have an *Adoxa-type* of embryo-sac. In *T. divaricatum* Guignard (1885) had previously reported *Normal-type*; recently Schaeppi and Steindl (1937) have confirmed this in *Osyris alba*, and Srinivasa Iyengar (1937) in *Santalum album*. Since neither Modilewski nor Schulle studied their plants thoroughly, their observations must now be regarded as doubtful.

Young (1923) stated that in the common potato the development of the embryo-sac is of the *Lilium-type* (*Adoxa-type*). Rees-Leonard (1935) and Lamm (1937) have corrected this and found a *Normal-type*. Since the three micropylar megaspores degenerate very early, they were missed by Young.

Miller (1919) reported that in *Zea mays* all the 4 megaspore nuclei enter into the formation of the embryo-sac. Weatherwax

(1919) and Cooper (1938) have, however, shown that a tetrad of megaspores is formed as usual and the lowest of these functions to form a *Normal-type* of embryo-sac.

McCollum (1939) has recently described the development of the embryo-sac of *Commelina angustifolia*. "It seems quite certain that all the four nuclei resulting from the meiotic divisions of the megaspore mother cell, enter into the structure of the embryo-sac. The lack of any evidence of walls separating the nuclei, also the lack of any evidence of disintegration of any of these nuclei points definitely to the same conclusion." In spite of the certainty in the author's mind there is nothing in his figures to convince a critical observer of the correctness of his statements. In another sp. of the same genus *C. benghalensis*, Maheshwari and Singh (1934) have already demonstrated a *Normal-type* of embryo-sac and more recently Lakshmi-Narasimhamurthy (1938) has done the same in several other members of the family.

Miss Duthie's work (1930) on *Lilium tigrinum* escaped my notice on the last occasion. She has reported an *Adoxa-type* of development. This is obviously incorrect, for in this species as well as many others belonging to the same genus, the *Fritillaria-type* has now been clearly demonstrated by Cooper (1935). The error is due to Miss Duthie's ignorance of Bambacioni's work on *Fritillaria*.

Joshi (1937) has recently investigated the embryo-sac of *Aloe vera* and found that the development is not of the *Adoxa-type* as supposed by Gioelli (1930) for several species of the genus. A normal tetrad of megaspores is formed of which the lowest functions and gives rise to a monosporic 8-nucleate gametophyte. Schnarf and Wunderlich (1939) have also contradicted the account of Gioelli, which was already regarded as very doubtful (Maheshwari, 1937).

Mauritzon (1936) thought that in *Costus igneus* the development is very probably of the *Adoxa-type*. Banerji (1940) has recently published an account of the life-history of another species, *C. speciosus*, and finds that the embryo-sac develops according to the *Normal-type*, the only variable feature being the arrangement of the megaspores which frequently form more or less isobilateral tetrads. Mauritzon's observations which are rather fragmentary, seem to be incorrect.

CONCLUSION

A review of all the known types of embryo-sacs leads one to the conclusion that the monosporic 8-nucleate type is the most primitive and the one from which all the others may be derived with the least difficulty.

The relationship of the *Oenothera-type* to the *Normal-type* is easy to understand. In the origin of the former the chalazal half of the embryo-sac has entirely disappeared and there is one division less so that no more than 4-nuclei are formed. A transitional stage is seen in the Lythraceae which display a reduction in the number of

divisions at the lower end of the embryo-sac. It is noteworthy that the four-nucleate monosporic embryo-sac has so far been found only in the *Oenotheraceae*, all other claims having been proved to be untrustworthy.

The *Allium*-type of embryo-sac is obviously the result of a failure of wall-formation during the second reduction division. Cases are known where rudimentary walls are laid down but soon disappear.

The remaining types of embryo-sacs are tetrasporic and are characterised by a complete lack of any permanent walls during megasporogenesis. The 16-nucleate condition (*Peperomia*-type) seen in a number of plants, belonging to diverse families, results from 2 further divisions after this stage. In the *Fritillaria*-type the number of divisions is the same but due to a fusion of the 3 spindles in the chalazal end, the primary four-nucleate stage is followed by a secondary four-nucleate one which gives rise to an 8-nucleate condition only after the next division. In the recently discovered *Plumbagella*-type the development stops at the secondary four-nucleate stage and the embryo-sac is organised with only one antipodal, 2 polar nuclei and an egg. *Plumbagella micrantha* is, as yet, the sole representative of this type of development.

In a very few plants of the *Plumbaginaceae* the 4 megaspore nuclei divide once to form 8, of which 4 remain arranged peripherally, one of them forming the egg, and 4 fuse in the centre. This type, first discovered in *Plumbago capensis* by Haupt (1934), is related on the one hand to the type found in *Penaea* and on the other to that in *Acalypha indica* (Maheshwari, 1940).

The *Adoxa*-type like *Plumbago* is characterised by 3 successive nuclear divisions from the megaspore mother cell to the egg-cell. The organisation of the mature embryo-sac is, however, different and more nearly resembles the *Normal*-type.

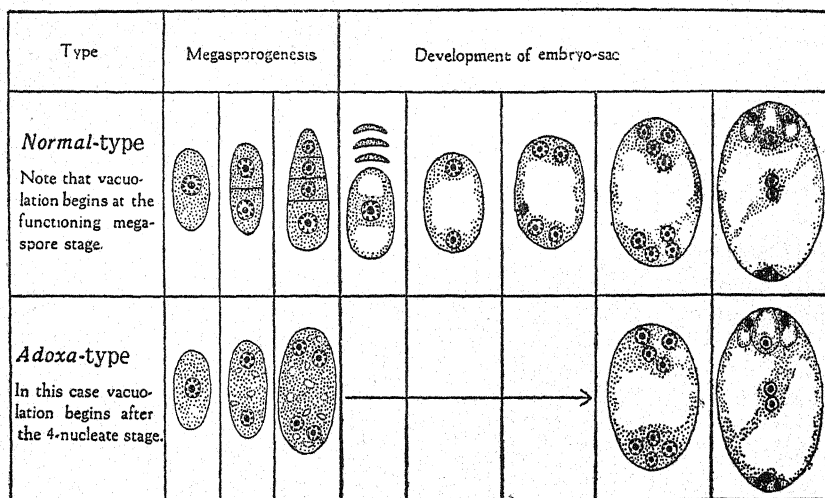
It would be interesting and helpful to note here the chief kinds of traps and pitfalls into which embryologists have frequently fallen.

The early disappearance of the 3 antipodals has been responsible for a number of reports of 5-nucleate (before polar fusion) and 4-nucleate (after polar fusion) embryo-sacs, although all the 8 nuclei are actually present in the beginning. Some 16-nucleate embryo-sacs have also passed as 8-nucleate because of the disappearance of a number of antipodal cells.

The degenerating megaspores which sometimes persist for a long time have been responsible for a few mistakes in the opposite direction. A good example is the embryo-sac of the *Oenotheraceae*, which for a long time passed as an 8-nucleate one, because of the persistence of the cells at the chalazal end which look very much like antipodals but are really outside the boundary of the embryo-sac.

Sometimes the nucellar cells or nuclei may get incorporated in the sac as a result of poor technique.

There are several examples of perfectly normal monosporic embryo-sacs having been mistaken as bisporic or tetrasporic through insufficiency of material or a somewhat too keen desire to find something deviating from the normal course of development. If in Fig. 3 under the *Normal-type* stages 2 and 3 and the degenerating megaspores in 4 are overlooked, the embryo-sac would easily pass as a tetrasporic one. For a critical observer another test is, nevertheless, available. The 2- and 4-nucleate stages of a monosporic embryo-sac always show a large central vacuole, while this is hardly ever the case in the tetrasporic embryo-sacs, where such a large vacuole is seen only after the 4-nucleate stage is over.



Figs. 3.—Diagrams showing the difference in development between a monosporic 8-nucleate and tetrasporic 8-nucleate embryo-sac. Note the difference in vacuolation between the 4-nucleate stages of the two types.

In conclusion, I am tempted to quote a few sentences from Dr. Johansen's recent book on *Plant Microtechnique* (p. 477):—

“In perhaps no other field of botanical investigation are there so many opportunities for making erroneous interpretations as during the development of the megagametophyte. As an instance there might be cited the case of *Lilium*, whose development was intensely investigated for many decades, yet it was not until a few years ago that the correct sequence of events was described. In so far as the purely technical aspects are concerned, sources of error may arise because of inadequate fixation, poorly differentiated stains, but above all from using too thin sections. The last fault is the most prevalent one ; many embryologists habitually cut sections

as thin as 5μ and then attempt to follow out the course of events by means of reconstructions. It is a far better procedure to determine the approximate diameter of the megasporocytes, megaspores, and megagametophytes at their various stages of growth and to adjust the thickness of the sections to correspond to each stage. The megagametophyte of *Lilium* during meiosis averages between 30 and 36μ in diameter; consequently the optimum thickness for sections at this stage is 24μ ."

Several notable improvements have been made during recent years in embryological technique, by which quicker and more precise results can be obtained. These will be dealt with at a later date in a separate paper.

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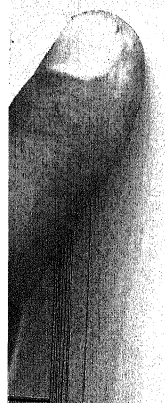
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THE LIFE-HISTORY OF MORINGA OLEIFERA LAMK.

By V. PURI, D.Sc.

Meerut College, Meerut

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As mentioned in an earlier note (Puri, 1934), Rutgers (1923) made some astonishing observations with regard to the development of the female gametophyte and the embryo in *Moringa oleifera*. His interpretations have already been shown to be based on insufficient data. It is now proposed to describe the morphology of this plant in some greater detail.

Collections of the material were begun at Agra in 1933 and continued at Meerut at different times and from different plants. Of the various fixatives used, Nawaschin's fluid (Chamberlain, 1934, p. 388) gave the most satisfactory results. Sections were cut 5-12 microns thick. For staining, largest use was made of iron-alum-haematoxylin followed by picric acid for differentiation (Maheshwari, 1933).

EARLY DEVELOPMENT OF THE ANTHER

In each flower there is an inner whorl of 5 perfect stamens followed by an outer whorl of 5-3 anther-less staminodes. The anther starts its development as a homogeneous mass of meristematic cells surrounded by epidermis. To begin with its outline is more or less circular in cross-section but soon it becomes oval and then faintly 4-lobed. The archesporial tissue differentiates at only two inner lobes instead of the usual four (Fig. 1). The result is a bi-locular anther, the like of which has also been reported in *Elodea* (Wyllie, 1904), *Styphelia longifolia* (Brough, 1924), *Phoradendron* (Billings, 1932), *Wolffia* (Gupta, 1935), *Aeginetia indica* (Juliano, 1935 a), *Vallisneria* (Witmer, 1937) and others.

In a few flowers, collected late in the season, some of the anthers showed four complete sporangia while in some others the extra pair was somewhat smaller than the other. In such flowers some of the staminodes, which usually end blindly, were also seen bearing a conical structure at their tips.

With regard to the evolutionary sequence of the bi-sporangiate and the more common tetra-sporangiate condition, Campbell (1897) has expressed the opinion that in the genus *Naias*, where all stages from one to four loculi may be seen in the anthers, the single loculus represents the primitive condition and that a plural number is formed by its subdivision. Recently, Witmer (1937) has adduced evidence in support of this opinion from his study of *Vallisneria*

spiralis. In this species the sporogenous tissue of both the stamens originates as one common mass, but later on, as the two stamens become distinguishable by a depression of the epidermal layer in the centre, a sterilisation of the hypodermal archesporial cells in that region results in two groups. A second sterilisation process divides each of the latter into two parts, which represent the two loculi of an anther. In the same way, further sterilisation may produce a tri- or tetra-locular condition.

In *Moringa* it seems more likely that the bi-sporangiate condition is derived from the tetra-sporangiate one. This is indicated by gradual reduction of loculi from 4 to 2 in a few cases mentioned above.

THE TAPETUM AND THE SPOROGENOUS TISSUE

The sporogenous tissue becomes clearly distinguishable only after one or two wall layers have been cut off (Fig. 1). It is now seen in a transverse section of the anther as a plate of about a dozen cells with deeply staining cytoplasm and somewhat prominent nuclei. The wall cells undergo several periclinal and anticlinal divisions till about 4-6 layers are formed on the sides.

The innermost of the wall layers differentiates into the tapetum which, as usual, attains the climax of its development about the time of reduction division in the spore mother cells. The tapetal cells are mostly 2-nucleate, but some, especially the larger ones, may show a greater number of nuclei. Fig. 2 shows a tapetal cell with the nucleus in telophase. I have never come across any amitotic divisions but during later stages the nuclei of a cell fuse and merge into a single nucleus which presents a bi-, tri-, or a multi-nucleolate appearance, probably depending on the number of nuclei taking part in its formation (Fig. 3). Raghavan (1938 *a*) has also reported such a fusion of nuclei in the tapetal cells of *Gynandropsis pentaphylla*. According to him the "fusion follows so quickly upon the division that it is hard to find them in a separate condition". Bhargava (1936) and Singh (1936) have also recorded the fusion of tapetal nuclei in *Chenopodium* and *Ranunculus* respectively. Cooper (1933) has made a detailed study of the behaviour of the tapetal nuclei in 43 species of Angiosperm and he has reached the conclusion that in some cases the nucleus divides more than once and, depending upon the incompleteness or completeness of the division, the mature cell is uni-, bi-, or pluri-nucleate. Evidently he does not believe in the fusion of the tapetal nuclei.

The tapetal cells on the inner side of the loculus are always 2-3 times more elongated radially than those on the outer side (Fig. 5). They contain a larger number of nuclei and comparatively bigger vacuoles. A similar difference in the size of the tapetal cells on the two sides has also been reported in *Scrophularia nodosa* (Coulter and Chamberlain, 1903), *Lactuca sativa* (Gates and Rees, 1921) and *Salvia mellifera* (Carlson and Stuart, 1936). In *Lactuca sativa* it

is further reported that the elongated cells are 4-nucleate, while the smaller ones generally contain only two nuclei.

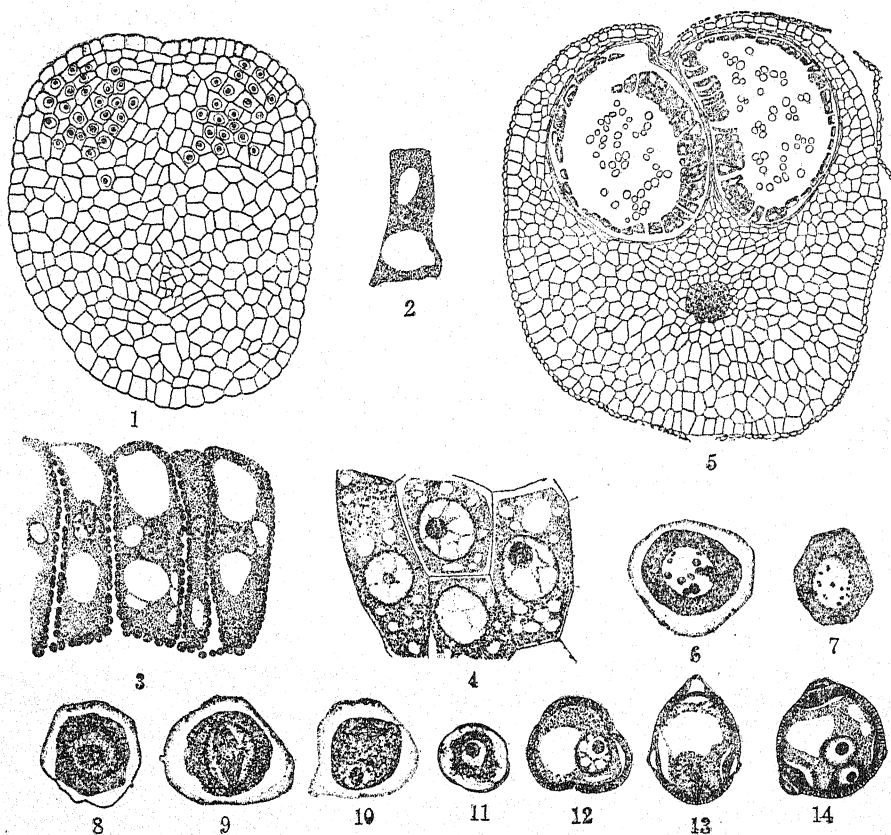
About the time the microspores are formed, a large number of small globules is seen on the inner and radial walls of the tapetal cells and in smaller quantities on the cell walls of the middle layers as well. Quite often they have been observed to lie inside the loculus and adjacent to the microspores and give the same staining reaction as the exine. Gorczynski's observations (1935) on *Cardamine chenopodiifolia* seem to be of much interest in this connection. His fig. 57 shows that the exine first begins to develop on that side of the pollen grain which is towards the tapetum. Later on, however, it extends on all sides (his figs. 56 and 58). Kosmath (1927), who has also observed such globules in several plants, concludes that chemically this substance is similar to cutin and gives the same staining reaction as the exine of pollen grains.

FORMATION OF MICROSPORES

Simultaneously with the development of the tapetum the sporogenous cells show an increase in size as well as in number. Finally the divisions cease and the cytoplasm of the cells begins to round up to some extent. At this stage, the nucleolus of the mother cells shows a number of dark spherical bodies within it. In one of the nucleoli in Fig. 4 thirteen such bodies can be counted. I am not in a position to make any statement on the nature of these structures.

The mother cells now enter upon the prophase of the reduction division. The nucleolus stains very lightly and finally disappears. Simultaneously with this, some large spherical bodies make their appearance within the nuclear membrane. Thirteen such bodies can be counted in Fig. 6. In all probability they are the "pro-chromosomes" similar to those recorded by Raghavan (1938 b) in *Polanisia* and *Gynandropsis*. Kuhn (1929) is reported by Raghavan to have observed them in another species of the Cappariaceae, *Capparis spinosa*.

Subsequently true chromosomes take the place of the "pro-chromosomes". In Fig. 7 fourteen bivalent chromosomes can be counted scattered about in diakinesis. The nuclear membrane now disappears and its place is taken up by a densely staining zone of cytoplasm. Figs. 8, 9 show the metaphase and anaphase of the first division. The spindle is narrow and pointed and always develops within the dark zone mentioned above. Some of the spindles which were cut transversely confirmed the chromosomes count of 28. Patel and Narayana (1938), on the other hand, report that the diploid number is 30 ($28 + 2$ fragments). I could not make out any such fragments in my preparations. In anaphase stage some chromosomes were frequently seen lagging.



Figs. 1-14.—Fig. 1. T.s. of a young anther showing sporogenous tissue differentiating at only two places ($\times 330$). Fig. 2. A tapetal cell with nucleus in telophase ($\times 460$). Fig. 3. Tapetal cells showing small dot-like globules along the inner and radial walls. Note the lobed nuclei ($\times 330$). Fig. 4. A group of microspore-mother cells. Note the black bodies within the nucleoli ($\times 460$). Fig. 5. T.s. of a bi-sporangiate anther showing unequal development of tapetal cells on the inner and the outer sides of the loculus ($\times 56$). Fig. 6. Microspore-mother cell showing "prochromosomes" prior to first reduction division ($\times 460$). Fig. 7. The same in diakinesis with 14 bivalents ($\times 460$). Fig. 8. Metaphasic spindle in polar view ($\times 460$). Fig. 9. Anaphase stage ($\times 460$). Fig. 10. Metaphase spindles of second division ($\times 460$). Fig. 11. One-nucleate pollen grain with nucleus in the centre ($\times 460$). Fig. 12. The same with nucleus pushed to one side ($\times 460$). Fig. 13. Telophase of the first division of the microspore nucleus ($\times 460$). Fig. 14. The 2-celled pollen grain ($\times 460$).

After the chromosomes have reached the poles, each group organises into a telophase nucleus. The nuclear membrane and one or two nucleoli now make their appearance. With the stain that I used (iron-alum-haematoxylin), most of the available space within

the nuclear membrane appears at this stage to be occupied by the nucleolus.

No partition wall is formed between the daughter nuclei, which promptly enter upon the second division. The spindles may lie in the same plane or at right angles to one another (Fig. 10). In early telophase the chromosomes form a more or less confused mass in the centre of the nucleus. According to the positions of the spindles the tetrads may be tetrahedral, isobilateral or quadrate. It may be of some interest here to record that while all the mother cells of a loculus generally show the same stages, there is invariably some difference in the degree of their development, in the two loculi of the same anther. For instance, if one loculus shows the metaphase of the first division, the other may show the telophase of the second division or even the tetra-nucleate condition. In some cases a slight difference has also been noted in the upper and lower ends of the same loculus.

During recent years a good deal has been written upon the behaviour and ultimate fate of the wall of the microspore-mother cells. Among others, Gates (1925) and Castetter (1926) are of the opinion that the mother cell wall always remains in tact and that within this a thick gelatinous wall is secreted by the cytoplasm. C. H. Farr (1916) and W. K. Farr (1920), on the other hand, are strong exponents of the view that the mother cell wall actually rounds off with the rounding of the protoplast and ultimately gelatinises to form a thick covering for the latter. In *Moringa* the mother cell wall does not round off nor does it separate from the adjacent walls except at the corners. In preparations counter-stained with fast green it is not difficult to distinguish it from the gelatinous layer on its inner side. The latter becomes more and more dense towards the periphery. Beer (1911) also draws attention to this difference in the density of the gelatinous wall in *Ipomoea* but believes that the outer portion is formed by the gelatinisation of the mother cell wall while the inner portion is secreted by the cytoplasm itself. In many cases an apparently empty space was observed between the primary wall and the gelatinous layer and in other cases between the latter and the protoplast. These appearances are probably the results of bad fixation.

Cytokinesis occurs through furrowing and it appears to take place very rapidly. Wedge-shaped projections make their appearance in the gelatinous wall and pierce into the protoplast. As a result the mother cell divides into four equal parts, its wall disappearing after a short while.

THE MALE GAMETOPHYTE

The separating microspores are more or less triangular in form (in sectional view) but they soon round off and lie scattered in the loculus. The nucleus, which is surrounded by a layer of dense cytoplasm (Fig. 11), is pushed towards one side of the wall (Fig. 12).

Fig. 13 represents the telophase spindle whose one end lies very close to the periphery. The two resulting cells are consequently very unequal in size (Fig. 14). The question whether the formation of a cell plate in this division is also followed by a real cell wall still remains an open one. Wulff and Maheshwari (1938) have already discussed the present state of our knowledge very adequately. In my own preparations I have not been able to make out any definite cell wall; what is actually seen is a clear space separating the generative cell from the vegetative one. A little later the nucleus of the generative cell appears to enlarge, the clear space between the two cells is no longer distinguishable and the generative cell penetrates, as it were, into the vegetative plasm. In some aceto-carminic smears I have observed that the generative cell divides within the anthersac so that the mature pollen grain is 3-celled.

The mature pollen grain is oblate spheroidal in form. The exine becomes moderately thickened and is finely but faintly granular. There are usually three clear-cut germ-pores situated equidistantly from one another in long, tapering furrows whose margins are not sharply defined. The intine is rather thin but becomes considerably thickened in the regions underlying the pores. The size of the mature pollen grains varies from 20-25 microns. In one exceptional case one very large 1-nucleate pollen grain was observed with five visible germ-pores.

THE OVULE

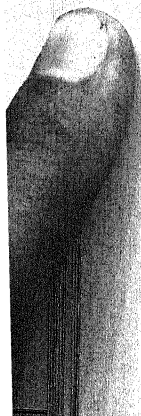
The young ovule appears as a small protuberance consisting of a mass of homogeneous meristematic cells. At a very early stage, as a result of quicker growth of cells on one side, it begins to bend over to the opposite side (Fig. 15). By further growth in the same direction it becomes fully anatropous with its micropyle directed upwards.

MEGASPOROGENESIS

Usually one, but sometimes 2-3 archesporial cells may differentiate in the hypodermal region when the ovule is still very young. They have deep staining cytoplasm and prominent nuclei, and may lie in the same line or parallel to one another (Fig. 16).

The two integuments appear almost simultaneously at the time of differentiation of the archesporium. Both of them remain entirely free from one another and also from the nucellus. Just as in the Capparidaceæ (Mauritzon, 1934) and Tovariaceæ (Schürhoff, 1926), the outer integument develops more quickly than the inner and on the side opposite to the funiculus it grows as a hood over the part of the micropyle formed by the inner integument. Consequently the micropyle presents not a straight but a zig-zag passage to the embryo-sac.

At the time of maturity of the embryo-sac the free tip of the inner integument becomes somewhat swollen on account of the



enlargement of its cells in that region and their becoming more or less papillose at their free ends. These cells are full of deep-staining cytoplasm and appear to be glandular in nature. After fertilisation the cells of the inner epidermis of the inner integument form a tapetum around the embryo-sac by becoming radially elongated and having deep staining cytoplasm. Usually they divide anticleinally but some of the cells may also undergo pericleinal divisions, resulting in two or three layers of deep staining cells (Fig. 35).

It may be interesting to record the occurrence of chlorophyll in the cells of the two integuments as well as the chalazal. As far as I know, our information on this point is very scanty. Hofmeister (1861, quoted in Schnarf, 1929) reported this, perhaps for the first time, in *Brunsvigia minor* and *Amaryllis belladonna*. Somewhat later Treub (1879, quoted in Schnarf, 1929) found it in the cells of both the integuments of *Sobralia micrantha* and in 1898 Berg (quoted in Schnarf, 1929) reported its presence in the outer integument and a portion of the chalaza in *Gladiolus communis*.

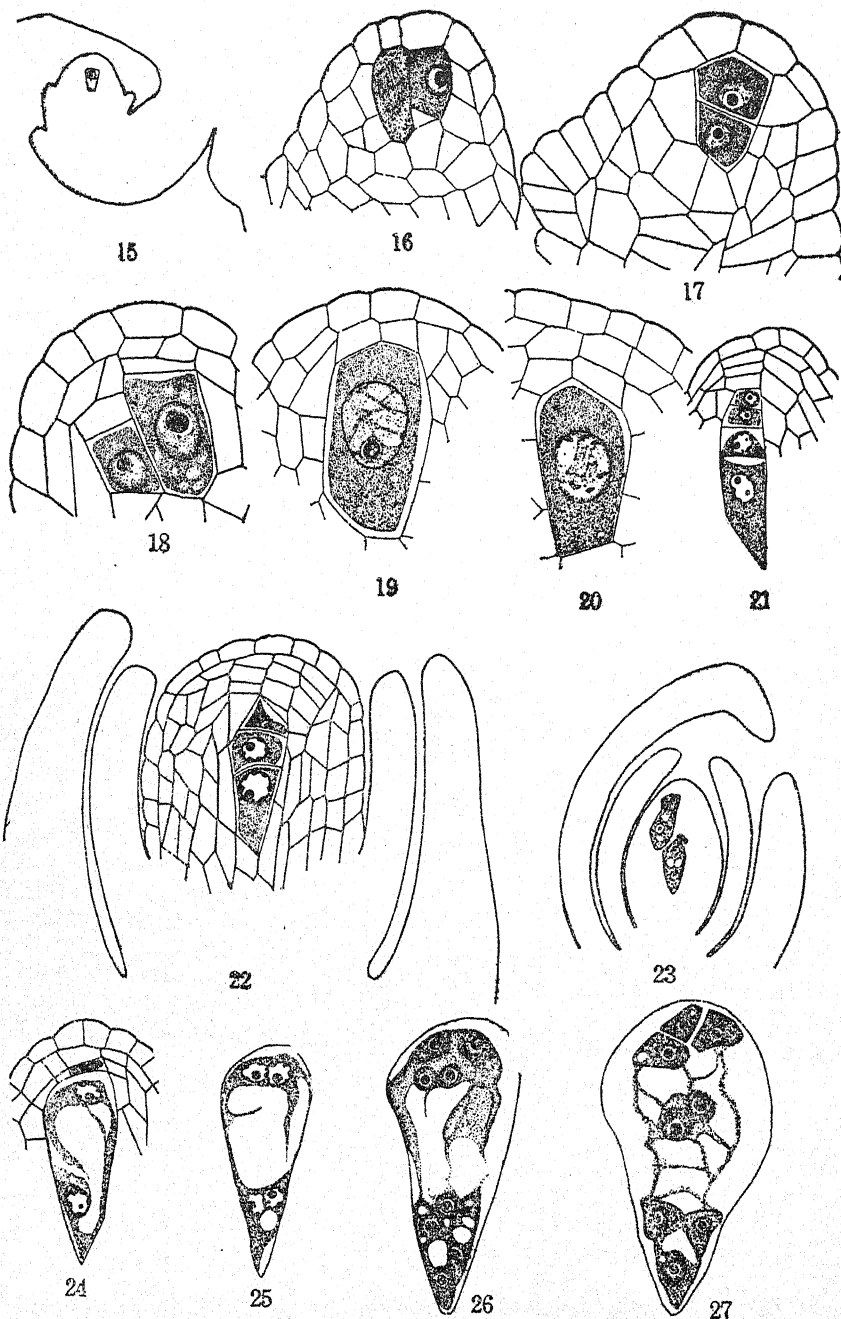
The archesporial cells divide in the usual manner to produce the primary wall cell and the megaspore mother cell (Figs. 17). The former by repeated pericleinal and anticlinal divisions forms 2-3 or even 4 layers of parietal tissue (Figs. 20-22). Rutger's statement that the archesporial cell does not divide and functions directly as the embryo-sac mother cell seems to be incorrect (see also Dahlgren, 1927 a). One of his own figures (No. 2) also raises some doubt as to the validity of his statement.

The megaspore mother cell then increases in size and prepares for the reduction division. Fig. 20 shows it in the diakinesis stage. As a result of two divisions a tetrad of four megaspores is produced which may either be linear (Fig. 21) or T-shaped (Fig. 22). Rutgers found only T-shaped tetrads; in my material both types are equally common.

Mauritzon (1934) remarks that in the Capparidaceæ a thin and long nucellus generally contains a linear tetrad while a small but broad one has a T-shaped one. Evidently he is explaining the occurrence of these two types on the basis of the available space. In *Moringa* both the conditions are met with perhaps because the nucellus here is neither very thin nor very broad!

DEVELOPMENT OF THE EMBRYO-SAC

After the tetrad divisions there is a rapid enlargement of the chalazal megaspore accompanied by an early degeneration of its three sister cells. Polarity is established after the first division of the nucleus and a large central vacuole separates on nucleus at each end (Fig. 24). The next two divisions result in the normal 8-nucleate embryo-sac. As is clear from Fig. 26, cell walls appear first around the antipodal nuclei, then the synergids and last of all the egg.



Figs. 15-27.—Fig. 15. Diagram of ovule at mother cell stage to show curvature ($\times 56$). Fig. 16. Vertical section of a young nucellus showing

ORGANISATION OF THE MATURE EMBRYO-SAC

The Egg Apparatus.—The three cells composing the egg apparatus are usually quite normal. The synergids possess large prominent hooks and faint striations of cytoplasm in the upper part. In some cases one or both the synergids were observed to assume the egg-like appearance by changing the relative positions of the nucleus and the vacuole. Cases of this type have been reported by many authors in different families (see among others Joshi, 1936; Johri, 1936; Puri, 1939). The egg hangs down to a lower level than the synergids and its nucleus lies adjacent to the lower limiting membrane.

The Polar Nuclei.—They are the biggest of all the embryo-sac nuclei. They meet in the middle and then ascend together and finally come to lie just beneath the egg apparatus. Although closely adpressed together they do not fuse until after fertilisation when the second male nucleus comes to lie by the side of one of them.

The Antipodal Cells.—There are three antipodal cells contained in the conical chalazal end. They usually degenerate very early and this is perhaps the reason why Rutgers considered the embryo-sac to be 5-nucleate. In some cases, however, I could distinguish their remains even after fertilisation.

It may be worth while at this stage to describe the changes which take place in the form of the embryo-sac. At the 2-nucleate stage (Fig. 24), it is broader at the micropylar end and tapers downwards. It continues to be like this up to the free 8-nucleate stage when the embryo-sac appears as more or less conical with its apex directed down. Henceforth the centre of enlargement begins to shift towards the chalazal end. A tendency to this effect is noticeable in Fig. 27. After some time the embryo-sac becomes broadest in the middle and tapers somewhat at the two ends. This condition persists for some time after fertilisation and then is again followed by a more or less conical form resulting out of an excessive enlargement of the chalazal end. In some cases the micropylar part becomes very narrow and the embryo-sac appears more or less flask-shaped with a very long and narrow neck. One such case is shown in Fig. 35 where a little bit of collapsing of the inner integument has added to the effect.

two archesporial cells lying parallel to each other ($\times 700$). Fig. 17. The archesporial cell has divided into a wall cell and a megaspore-mother cell, slightly oblique ($\times 700$). Fig. 18. Two megaspore-mother cells lying parallel to each other ($\times 700$). Figs. 19-20. Megaspore-mother cells in preparation of the division ($\times 700$). Figs. 21-22. Linear and T-shaped tetrads of megaspores ($\times 440$). Fig. 23. Vertical section of an ovule showing unequal growth of the outer integument and two mother cells functioning ($\times 56$). Figs. 24-25. 2- and 4-nucleate embryo-sacs ($\times 440$). Fig. 26. 8-Nucleate stage showing formation of antipodals ($\times 440$). Fig. 27. Same showing differentiation of cells at micropylar end also and the meeting of polar nuclei ($\times 440$).

The freshly organised embryo-sac contains starch grains of varying sizes. They are especially abundant at the time of fertilisation when they occur even in the cells of the egg apparatus. According to Dahlgren (1927 *b*) the starch content of the embryo-sac reaches its maximum shortly before fertilisation and from that time onward it diminishes more or less rapidly. He explains this on the basis of relative inactivity of the embryo-sac at that time.

POLLINATION AND FERTILISATION

Pollination is effected by bees which visit the flowers in large numbers in the morning hours. The two anterior petals form a suitable landing place for them. Engler and Prantl (1936) quote Gould as having noticed pollination in *Moringa oleifera* being effected by a bird (*Mellisuga minima* Gould) in Jamaica and St. Domingo. At Meerut I never saw any birds visiting the flowers in such large numbers as to be of much service in pollinating them on a large scale.

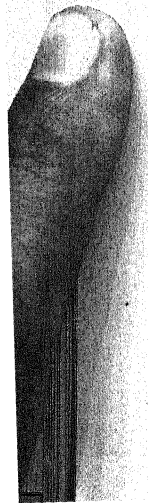
At the time of germination the pollen grain swells and the intine begins to protrude out of every germ pore. Later only one protuberance develops further and forms the pollen tube.

In some cases the pollen grains had begun to germinate *in situ*, although the tubes were hardly longer than the radius of the pollen grain. It was observed that the occasional muggy weather caused by winter rains encourages germination in the anther. Weinstein (1926) also states that in *Phaseolus vulgaris* many pollen grains begin to germinate within the anther and develop tubes of considerable length. Germination of pollen grains in the anther is, of course, well known in cleistogamous flowers (see Madge, 1929, on *Viola odorata* and West, 1930, on *Viola riviniana*; Erisendahl, 1929, on *Elatine*; Maheshwari and Singh, 1935, on *Commelina benghalensis*).

The stigmatic cells are very rich in cytoplasm and take a deeper stain than the cells lower down. The inner epidermis of the style also shows these features but not in such a high degree. The pollen tubes pass down along the wall of the canal, make their way through the micropyle and finally enter the embryo-sac by destroying one of the synergids.

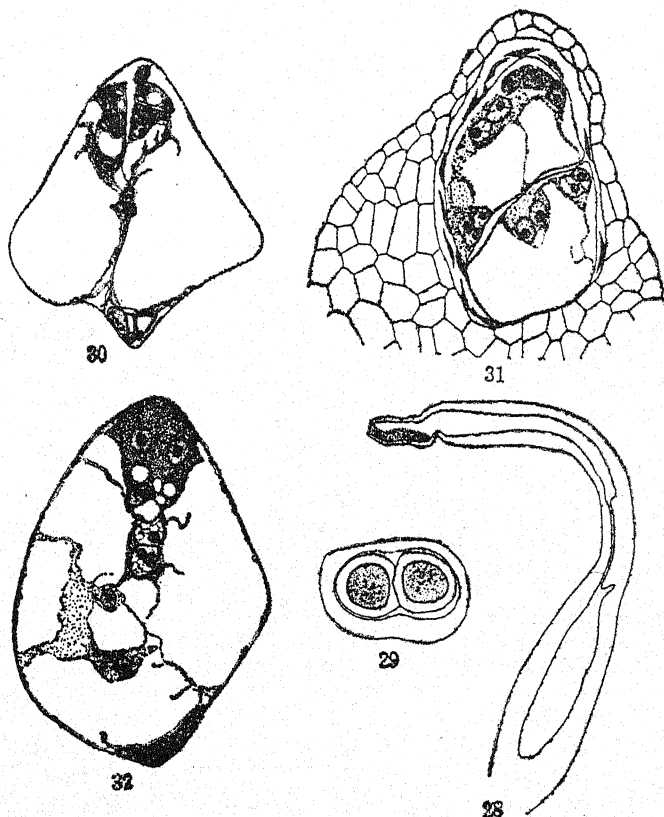
The hollow style opens directly to the exterior. The pollen mass deposited on its tip has often been observed to descend down into the interior of the stylar canal (Fig. 28). When I first observed this in my sections, I was led to think that some small insects (ants are often seen on flower parts) might have accidentally pushed it downward. Later, some flowers (on branchlets that were free from insects) were artificially pollinated and kept under a bell-jar. On dissection of these styles also the pollen mass was found to be present in the interior of the stylar canal.

Gravity is out of question in this connection; for, if it can work at all in this case, it must be in the opposite direction, since the



styles are bent downwards in open flowers. The only other explanation, therefore, is that there is some sort of suction mechanism, as was suggested by Sahni (1935-36) in connection with a similar report on *Butomopsis lanceolata* (Johri, 1935). Careful observations were made to find out if there was anything like the "pollination drop", of gymnosperms on the top of the style. I did not succeed, however, in finding an actual exudation.

One of the male nuclei discharged by the pollen tube enters the egg, lies by the side of its nucleus and ultimately fuses with it. The second male nucleus has been observed clinging to one of the polar nuclei before fusion. In both the fertilised egg and the primary endosperm nucleus more than one nucleolus can often be observed.



Figs. 28-32.—Fig. 28. Diagram of a vertical section of the pistil to show the pollen mass in the interior of the style ($\times 15$). Fig. 29. T.s. ovule showing two nucelli having a common outer integument but separate inner ones ($\times 26$). Fig. 30. Two mature embryo-sacs lying parallel to each other. The separating wall is visible only for a part of the distance ($\times 460$). Fig. 31. Two embryo-sacs lying one above the other ($\times 460$). Fig. 32. Mature embryo-sac having two extra pairs of nuclei. For explanation see text ($\times 460$).

SOME ABNORMALITIES

In one case two nucelli, each having its own embryo-sac and inner integument, were found to be surrounded by a common outer integument (Fig. 29). Both the nucelli have an independent vascular supply and the condition appears to have arisen out of a fusion of two separate ovules at a very early stage. Witmer (1937) has reached a similar conclusion with regard to double ovules he observed in *Vallisneria spiralis*. Cases of double nucelli have also been recorded in *Hartmannia* (Johansen, 1929) and *Hydrilla* (Maheshwari, 1936). Joshi (1936) has reported this to be very common in the family Lythraceæ.

Sometimes the same nucellus was seen to have two or more embryo-sacs lying parallel to each other or one above the other (Figs. 30-31). In either case it is equally likely that the different embryo-sacs may be derived from the products of the same or different mother cells. For instance, the condition illustrated in Fig. 30 can be obtained from the megaspores of two different tetrads as well as from those of the same tetrad after one of the megaspores has undergone a little sliding growth over the other. Similarly the condition shown in Fig. 31 can be derived from that depicted in Fig. 23 or from the megaspores of the same tetrad. But since more than one megaspore from a tetrad has never been observed to function it seems more likely that they have developed from different mother cells.

In some cases one, two or four additional nuclei were seen lying in the middle of the embryo-sac close to the polar nuclei. In the case sketched here in Fig. 32, there are four such nuclei, all practically of the same size. Of the various sources from which such extra nuclei can be derived the following two appear to be quite pertinent here.

In some cases the extra nuclei in the embryo-sac may be assigned to the surrounding nucellar cells which after disintegration may set free their nuclei into the latter. Such a condition has actually been observed in *Cocos nucifera* (Quisumbing and Juliano, 1927) and *Grevillea robusta* (Kausik, 1938).

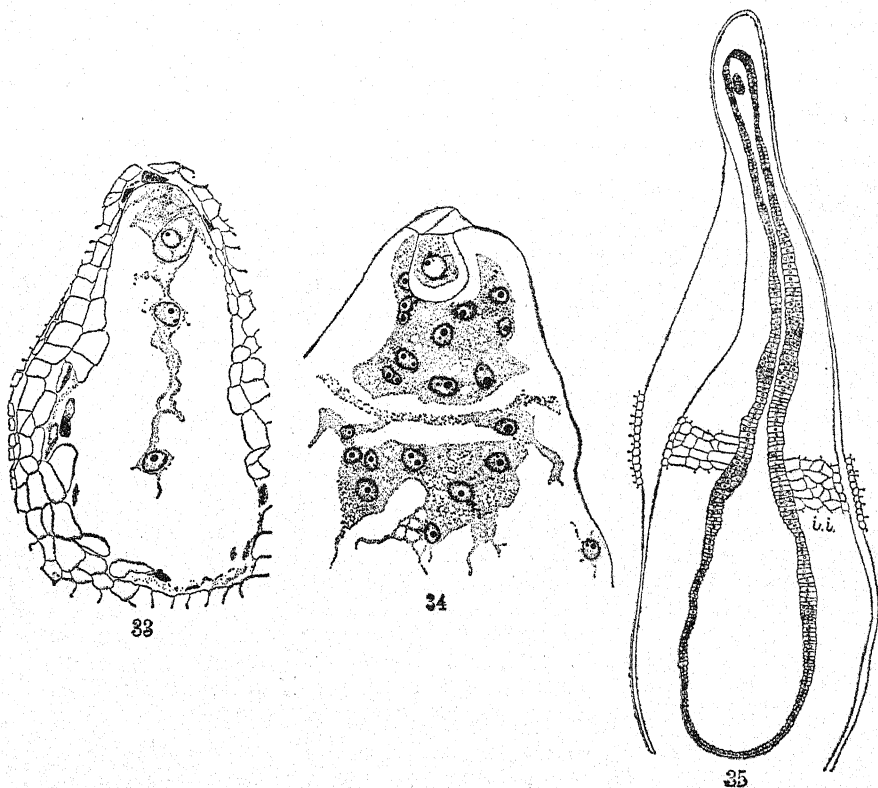
In other cases the additional nuclei may be due to the fusing together of two or more embryo-sacs of the same or different ages. On account of the fact that these nuclei are often larger than the ordinary nucellar nuclei, this appears to be the more probable cause. Frisendahl's (1927) drawing of a 16-nucleate embryo-sac in *Elatine* is a particularly clear case of the fusion of two gametophytes lying parallel to each other. Cases where fusion is not complete and the separating wall can be made out at places have been reported in *Reseda alba* (Oksijuk, 1929); *Anona* (Juliano, 1935, Fig. 25); and *Dysoxylum nutans* (Wiger, 1935, Fig. 25D, a, b).

Sterile Ovules.—It was surprising to find that in some ovaries all the ovules were sterile and their nucelli had protruded out of the inner integuments and come to lie in the micropyle. The place of

the embryo-sac was occupied by a very small mass of a brownish substance which probably represents the disintegrated remains of the sporogenous tissue if any. In his work on the *Onagraceæ*, Johansen came across many cases of sterile ovules. In *Gayophytum ramosissimum* (Johansen, 1932) "after the second division in the young megagametophyte, the three upper groups of chromosomes which would form the egg nucleus and the synergid nuclei respectively, are unable to reconstitute themselves into nuclei but become clumped together and finally break down completely". In *Moringa* the wholesale sterilisation of all the ovules in some ovaries appears to have occurred at a still earlier stage and the cause seems to be more universal than localized.

ENDOSPERM

The two polar nuclei lie close together but their fusion begins only after the arrival of the second male nucleus. After triple



Figs. 33-35.—Fig. 33. Embryo-sac with fertilized egg; endosperm binucleate ($\times 460$). Fig. 34. Portion of embryo-sac to show micropylar accumulation of endosperm nuclei around the egg. Note the gelatinous sheath separating the small micropylar chamber from the lower portion ($\times 460$). Fig. 35. Vertical section of an exceptionally narrow embryo-sac surrounded by integumentary tapetum ($\times 56$).

fusion, the primary endosperm nucleus undergoes a series of very rapid divisions. Fig. 33 shows the first two nuclei of the endosperm which lie quite free in the embryo-sac. For some time the divisions continue to be simultaneous but in later stages only neighbouring nuclei are seen in similar phases while there is a gradation of phases from one end of the embryo-sac to the other. As a rule a considerable aggregation of endosperm nuclei is seen in the micropylar end (Fig. 34) and a casual observer may entirely miss the egg in this 'crowd'. In some embryo-sacs the micropylar part of the endosperm was found to be separated from the larger lower portion by a transverse gelatinous partition (Fig. 34) but in other cases it was quite absent and I am unable to throw any light on its nature. Mauritzon (1935 a, Fig. 6, i) also describes and sketches a similar case in *Empleurum serrulatum* but does not say anything about its origin.

Cell formation begins at a very late stage in the micropylar end and is confined to that region. The cell walls are very indistinct and each 'cell' contains several nuclei. By the time the seed matures, the whole of the endosperm is used up and consequently the seed is non-endospermic.

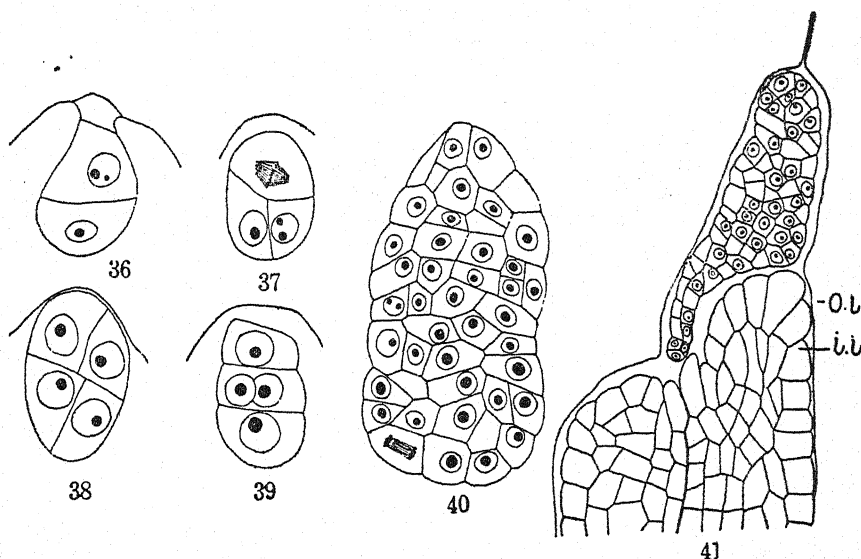
In some cases no egg could be detected at the micropylar end, nevertheless the endosperm was formed in the usual way. This led me to a dissection of mature seeds and as a result of this study it was found that about 15-20% of them have no embryos. Reports of similar type by Joshi and Rao (1934) and Wiger (1935) have, however, invited much adverse criticism [see particularly Singh (1934) for the first and Mauritzon (1935 b) for the second].

EMBRYO

The first division of the oospore is transverse and occurs when the fruit is already 3-4 inches in length and there are 3-4 hundred endosperm nuclei present within the embryo-sac cavity (Fig. 36). Such a late division has also been described for *Mangifera indica* (Maheshwari, 1934), where a thousand free nuclei may be found in the endosperm before the egg divides. The next division which always occurs in the apical cell is generally along the longitudinal axis of the embryo (Fig. 37). Sometimes, however, this is transverse and results in a linear row of three cells. In the former case the basal cell divides by a vertical partition and forms a quartet of four cells (Fig. 38). Fig. 39 shows another arrangement of the four cells constituting the pro-embryo. Further divisions are rather irregular and result in a bulbous embryo and a massive suspensor (Fig. 40).

A few interesting features have been noted regarding the position of the embryo. In some cases it gets pushed out of the endostome and comes to lie in the exostome (the part of the micropyle formed by the outer integument). In one case an unusually large embryo of a more or less irregular form was found lying in such a

position (Fig. 41). How it arose there or got squeezed out into such a position from the embryo-sac is not clear.



Figs. 36-41.—Figs. 36-40. Different stages in the development of pro-embryo and embryo ($\times 460$). Fig. 41. An embryo lying in the micropyle, outside the inner integument; an exceptional position ($\times 160$).

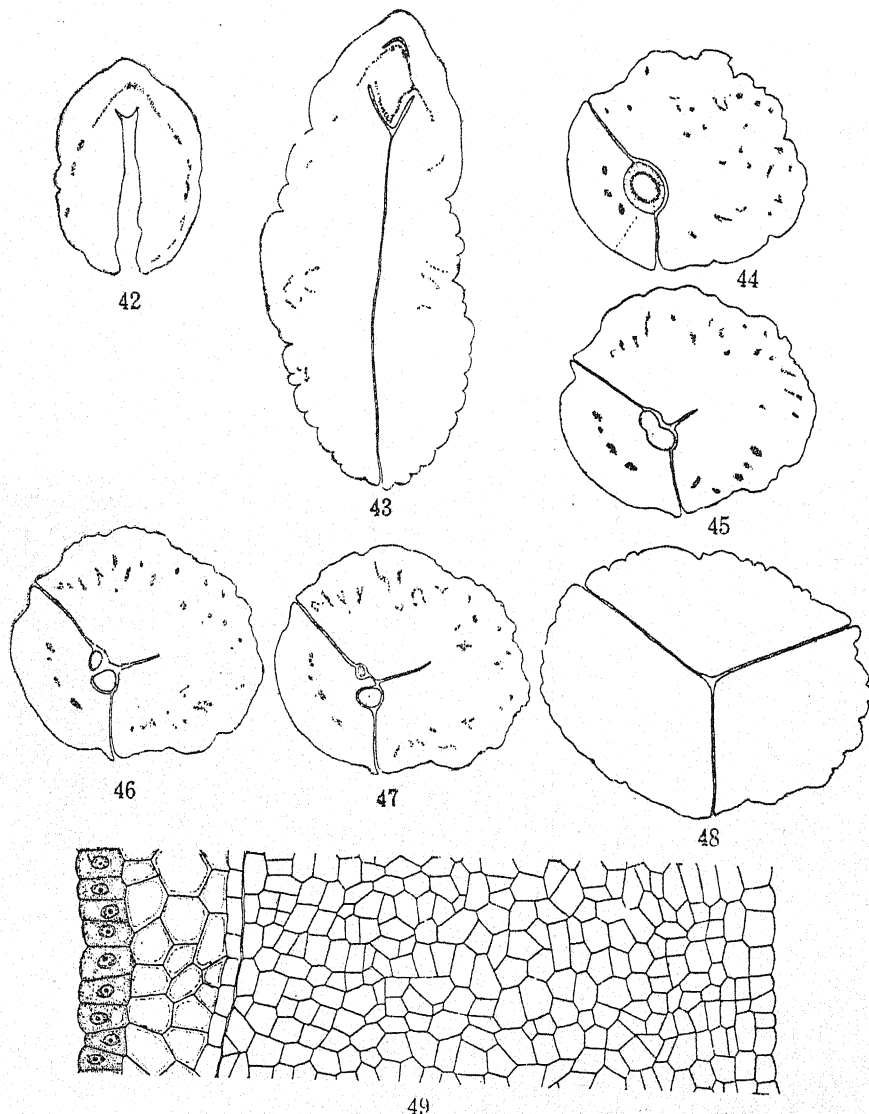
With further growth the globular embryo becomes bilobed at the lower end due to cell divisions being restricted to its periphery. The radicle, plumule and the two cotyledons are now differentiated in the usual way (Figs. 42, 43). Generally the two cotyledons are somewhat unequal in width and the larger one, although single near the hypocotyl, becomes bilobed downwards (Figs. 44-48). This tricotyledonous condition is apparently a case of schizocotily. In Fig. 44 the two cotyledons are very unequal in size. The smaller cotyledon on the left receives only three vascular traces while the larger one gets just double their number. This may give the impression that the latter is a compound structure formed by the coalescence of two cotyledons. It must be pointed out, however, that the three traced condition is not the rule in the species, the number of traces a cotyledon receives apparently depending upon the size of the cotyledon. If both of them are more or less equal they may receive 4-5 traces each.

As remarked by Compton (1913), the phenomenon of poly-cotily is extremely rare in angiosperms, many of the reputed examples being simply instances of deeply bifid cotyledons. However, it is known to occur in certain species of *Persoonia* (Proteaceæ), *Nuytsia* and *Loranthus* (Compton, 1913). Recently Johnson (1936) has reported several instances of tricotyledonous embryos in *Eugenia hookeri*.

Curiously enough, in some cases, the plumule also shows a bilobed condition at the tip, one of the lobes being much smaller than the other (Figs. 45-47). The ring of the procambial tissue also shows a corresponding split.

SEED

The mature seed is a white triangular structure measuring about an inch in length and $\frac{1}{8}$ " in thickness. It is provided with



Figs. 42-49.—Figs. 42-43. Vertical sections of embryos showing the differentiation of radicle, plumule and cotyledons ($\times 18$). Figs. 44-48. T.s. of an embryo showing an apparently tricotyledonous condition. For explanation see text ($\times 26$). Fig. 49. A portion of seed-coat from a young seed. Note the glandular layer formed by the inner epidermis of the inner integument on the left side ($\times 50$).

three wings running equidistantly along its length and produced at both ends beyond the main body of the seed.

The outer integuments consists of only 5-6 layers of cells at the time of fertilisation, but later on the number increases considerably due to meristematic activity which is specially noticeable in sub-epidermal layers all round. The cells of the outer epidermis are rectangular or squarish in form and show an increase in size towards the micropylar end. The inner epidermal layer, on the other hand, is not particularly different from the adjoining tissue (Fig. 49). At the three angles the outer hypodermal layers become specially active and soon form outgrowths which result in the wings.

The inner integument, which consists of only three layers to begin with, becomes 5-7 layered in a young seed. On the outer side it is delimited by small tangentially flattened cells which are very insignificant in the lower region but becomes radially elongated in the extreme micropylar end (Fig. 41). The inner epidermis, on the other hand, shows the reverse tendency. In the lower region it forms a prominent layer, the integumentary tapetum, whose cells are radially elongated and richly protoplasmic (Fig. 49). Towards the micropylar end these cells become smaller and smaller till they are of the ordinary size.

FRUIT

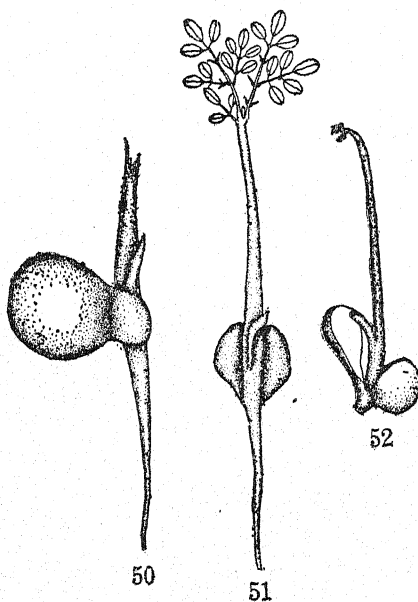
Soon after fertilisation, the perianth and stamens dry up and fall off so that nothing remains except the single slender pistil. At this stage the ovary wall consists of a homogeneous mass of parenchymatous cells delimited on both sides by radially elongated epidermal cells.

The ovary wall is 15-20 cells thick at the time of fertilisation but later on further increase is brought about by meristematic activity in some sub-epidermal layers. The intrafascicular cambium of the vascular bundles also becomes active producing more xylem than phloem. The cells of the periphery and specially those at the angles of the fruit become somewhat thickened while the rest remain parenchymatous throughout. True stomata with connecting intercellular spaces occur frequently in the outer epidermis. At intervals, the cells in the inner part of the pericarp become meristematic and form the parenchymatous tissue which fills the empty spaces between seeds. In dry fruits this appears as white flaky tissue surrounding the seed on all sides.

The mature fruit ("drumstick") is a three-sided pendant capsule measuring 9-18" in length. When young, it is twisted and has nine ribs running along its length. But at maturity three of them become more prominent and result in a triquetrous fruit. Dehiscence starts from the distal end and proceeds towards the base along the three angles.

SEEDLING

While germinating seeds to test their viability I came across a few seedlings which showed a small branch attached to the main shoot in the neighbourhood of the cotyledons. This branch was always found to be very small and appeared to be abortive. In one such case the main shoot and the little branch appeared to be emerging out of the cotyledons (Fig. 50). The tip of the former was injured in some way. In another case the small branch was given out from a point very close to the attachment of the cotyledons, but apparently it had no connection with the latter, that is, it was not growing in the axil of any of the cotyledons (Fig. 51). In the third case the branch was given out at a somewhat higher level (Fig. 52).



Figs. 50-52.—Young seedlings. For explanation see text.

Due to insufficiency of suitable material I could not determine the morphological nature of this "branch". It seems desirable to grow some seedling to an older stage in order to determine definitely whether the structure in question is a young compound leaf or a branch. In the latter case it may have originated from a bud in the axil of one of the cotyledons or alternatively by splitting of the plumule. Cotyledonary buds have been reported in quite a number of cases. Tiwari (1929) has described them in *Cassia tora*, *Cicer arietinum* and a few other plants. I myself have observed them in castor oil and mango. But here the fact that they have little connection with the cotyledons—i.e., do not arise in their axils—

goes against their being regarded as cotyledonary. Close examination of Figs. 45-47, on the other hand, appears to suggest that they may have originated by the splitting of the plumule.

SUMMARY

The stamens are bisporangiate. Some tetra-sporangiate anthers are also reported and it is suggested that the former is a derived condition.

The tapetal cells lying on the inner side are more elongated radially than those on the outer side. The larger cells usually contain three to four nuclei which fuse together in later stages. At about the time of the micropore formation small spherical bodies make their appearance along the walls of the tapetal cells. They show the same staining reaction as the exine.

The generative cell is separated from the vegetative cell by an apparently clear hyaline zone which disappears after some time.

The development of the embryo-sac is of the *Normal*-type, the three antipodal cells usually degenerating very early.

A case of double ovule and several instances of ovaries having all sterile ovules have been described and a number of supernumerary nuclei have been reported.

Pollination is effected by bees. The pollen mass was usually seen within the hollow style.

The first division of the fertilised egg is transverse and occurs when 3-4 hundred endosperm nuclei have been produced. A massive suspensor and a bulbous pro-embryo are formed. Whenever the cotyledons are of unequal size the larger of the two often splits up longitudinally into two giving a tricotyledonous appearance.

Micropylar accumulation of endosperm nuclei is frequently noticed. Wall formation in the endosperm is very irregular and occurs only in the micropylar end.

Seed, fruit and a few seedlings have also been described.

I have great pleasure in recording my sincere thanks to my teacher, Dr. P. Maheshwari, for the interest he took in this work throughout its progress. I am also grateful to Prof. R. R. Gates (London) for the loan of some literature and to Prof. Knoll (Vienna), Dr. Wodehouse (Yonkers), Sir Arthur W. Hill and Dr. Metcalfe (Kew) for their valuable suggestions concerning certain points.

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THE GROWTH OF *AZOLLA FILICULOIDES* IN MINERAL SOLUTION WITHOUT ADDITION OF 'AUXIMONE'

BY AHMAD, GHILAS-UD-DIN, B.Sc. (AGRI.) (PB.), B.Sc. (LOND.),
M.Sc. (CALIF.)

Assistant Professor of Botany

Punjab Agricultural College and Research Institute, Lyallpur

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INTRODUCTION

THE water ferns, *Azolla* and *Salvinia* and the common Duck weeds, *Lemna minor* and *Lemna major* afford an attractive field to a plant physiologist for working out photosynthetic, salt accumulation, effect of various factors on growth and a score of other similar problems. This is particularly so, owing to the ease in maintaining constant environmental conditions during the period of the experiment and a more ready means of demonstrating their growth. Whereas light and temperature can be easily controlled, it will be necessary to grow these plants in mineral solution, the chemical nature of which is also exactly known. It is a fact that any addition of organic extracts will change the pH as was shown by Ashby¹ and thus the conclusions drawn will be influenced by more than one factors. Besides this great drawback, when you add extract from any organic matter, you do not know the amount and nature of all the constituents thus added. The author was interested in the water culture experiments of *Azolla filiculoides*. The object was to be sure of successfully growing *Azolla* sp. in mineral solution of known composition without the addition of peat or yeast extracts. If this could be done, it would naturally form a stepping stone for other interesting physiological work.

REVIEW OF LITERATURE

Bottomley² showed that the addition of extracts of 'bacterized' peat when added even in small quantities to wheat seedlings growing in water culture caused a marked increase in growth. Bottomley³ gave the name of 'auximone' (Gr. promoting growth) to these plant food accessories present in the peat extract. Later on, he⁴ conducted his experiments on *Lemna minor* in Dittmer solution and came to the conclusion that the presence of soluble organic matter is essential for healthy and complete growth of *Lemna minor*. An examination of the aqueous extract of 'bacterized' peat by Bottomley⁵ showed that it contained certain purine and pyrimidine bases, together with phosphoric acid. It was also successfully shown by the same author⁶ that the addition of the

derivatives from nucleic acid has a marked effect on the growth of *Lemna* plants in water culture and secondly that the nitrogen fixing bacteria can elaborate products which stimulate growth.

From the previous works already mentioned above, Bottomley definitely concluded that the addition of 'auximone' in the form of water extracts of 'bacterized' peat, etc., to the nutrient solution was absolutely essential for the proper and healthy growth of *Lemna minor*. He⁷ then tried Knop's solution besides Dittmer solution and grew both *Lemna minor* and *Lemna major*, and came to the conclusion that the requirements of both the *Lemna* sp. were identical in both the solutions.

In his⁸ last experiment of the series, he grew *Salvinia natans*, *Azolla filiculoides*, *Limnobium stoloniferum*, besides *Lemna major* and *Lemna minor* to try the effect of organic matter on the growth of these plants. His conclusions are that all these plants were unable to maintain themselves in health without the presence of organic growth-promoting substances.

His observations regarding *Azolla filiculoides* are interesting in this respect that these plants, in the mineral solution only, retained their healthy appearance to a much longer extent than in the case of the plants with which the previous trials were carried out, probably on account of the symbiotic relationship with endophic anabæna. Mockeridge¹³ was the chief supporter of the 'auximone' theory and she¹⁴ tried to refute the various objections against it after the death of Professor Bottomley. Mendiola¹² by growing *Lemna minor*, in modified Pfeffer's solution for months was the first to question Bottomley's theory of 'auximone'. Clark and Roller¹⁰ criticized the theory and successfully grew for months Duck-weed in a physiologically balanced mineral solution. They came to the conclusion that the function of organic matter in the nutrition of green plants might be to increase the speed of reproduction and growth, rather than serve as an essential constituent.

Saeger¹⁸ in dilute ordinary culture solution grew *Spirodela*, *Lemna* and other water plants. Wolfe²⁰ pointed out that Bottomley's mineral solutions were not physiologically balanced, hence it did not promote normal growth. He even suggested that the term 'auximone' might be dropped from the literature altogether.

Ashby¹ grew *Lemna* under controlled conditions in dilute salt solutions alone and also with the addition of water extracts of horse dung. His conclusions are that whereas *Lemna* could grow in culture solutions of pure mineral salts indefinitely, the addition of organic matter resulted in an increase in frond area and cell size over the controls.

THE EXPERIMENTAL TECHNIQUE

Healthy and uniform springs of *Azolla filiculoides* were carefully selected from a tank and placed in clean tank water before using them for various experiments.

Fresh weight of the plants was taken as the criterion of growth. Hoagland and Broyer's¹¹ method of finding fresh weight by centrifuging at the rate of 400 revolutions per minute for five minutes was successfully employed. For the details of this method original paper should be consulted.

The plants were grown in one quarter strength of Hoagland solution¹¹ both with and without water-soluble extracts of 'bacterized' peat and Fleischmann's yeast. It was found much better to use yeast extracts, which were obtained by grounding weighed quantities of yeast with sand in water. The mixture was first filtered through a fine cloth and then it was centrifuged twice for five minutes at 4000 revolutions per minute and filtered again. This extract was added to one litre of quarter strength Hoagland solution in the required doses. The peat extract was also similarly treated and used. The solution was changed once a week. The plants as a control were also grown side by side in the tank water. The beakers were wrapped on the sides with a black cover to cut off light.

The plants were grown both in the green-house and under controlled conditions of light, temperature, etc.

The temperature of the baths was controlled by mercury thermo-regulators and the beakers containing plants were placed inside clay pipes, two feet in height and eight inches in diameter. These pipes had series of holes both towards the bottom and the top so arranged that water and air could circulate freely and keep the temperature of the plants constant within a range of $\pm 2^{\circ} \cdot 0^{\circ} \text{C}$.

The light was supplied for sixteen hours daily by the gas-filled Mazda lamps placed on the upper side of the pipes in dome-shaped covers. A Hartfold electric time switch of 220 volt and 50 ampere capacity was employed for controlling the daily illumination period. The light intensity was measured in some cases with a single phototropic cell, in foot-candles under experimental conditions.

Note.—In all the experiments described below, by the mineral solution is meant one quarter strength Hoagland solution.

THE DATA

Experiment I.—The *Azolla* plants were grown in eight different media for a period of fifteen days in the green-house. The weather was bright and clear throughout the experiment. The temperature of the green-house was throughout approximately 20°C . The atmosphere was free from dust. The fresh weight of the plants was noted both in the beginning and at the end of the experiment.

The following Table I shows:—

- (a) The details and amounts of the yeast and peat from which extracts were obtained by grounding the material with sand and water and then centrifuging and filtering.
- (b) The fresh weight of the plants at the beginning and at the end.
- (c) The per cent. increase in fresh weight in a fortnight.

TABLE I

Shows the Increase in Weight of Azolla filiculoides in Different Media in a Fortnight, when kept in the Green-house at $20^{\circ} \pm 2^{\circ}$ C.

Serial No.	The media used	Fresh weight		Per cent. increase	Remarks
		Initial	Increase in two weeks		
		gm.	gm.		
1	The mineral solution <i>plus</i> aqueous extract of 6 gm. of yeast	4.072	3.648	89.50	The plants grown with yeast extract showed more growth and bigger size, next in order were those supplied with peat extract.
2	The mineral solution <i>plus</i> aqueous extract of 3 gm. of yeast	4.025	2.725	67.70	
3	The mineral solution <i>plus</i> aqueous extract of 1 gm. of yeast	3.552	2.248	63.28	
4	The mineral solution with aqueous extract of 6 gm. of 'bacterized' peat	3.860	2.540	65.80	The plants in Hoagland solution and in tank water looked comparatively smaller in size.
5	The mineral solution <i>plus</i> aqueous extract of 3 gm. of 'bacterized' peat	3.90	2.40	61.54	
6	The mineral solution <i>plus</i> aqueous extract of 1 gm. of 'bacterized' peat	3.825	2.275	59.47	
7	The mineral solution	3.600	1.60	44.20	
8	Tank water	3.292	1.12	34.00	

Experiment II.—The plants were grown under controlled conditions of light and temperature. One set of beakers contained

GROWTH OF *A. FILICULOIDES* WITHOUT 'AUXIMONE' 289

the mineral solution only and to the other set extract from three grams of yeast per litre was added. These two sets were kept for a week under identical conditions of light and temperature.

TABLE II

Shows the Increase in Fresh Weight of Azolla filiculoides in One Week, when Cultivated under Controlled Conditions of Light and Temperature ($20^{\circ} \pm 2^{\circ}$ C.) With and Without Yeast Extract added to the Mineral Solution

Light Intensity	Per cent. increase in fresh weight in one week		Remarks
	Set I	Set II	
	with yeast extract	without yeast extract	
100 Watts	30.8	20.0	The set with yeast extract showed better growth.
500 Watts	99.8	91.5	Increased light intensity and the yeast extract was inductive to growth.

Experiment III.—After these two preliminary experiments, the cultivation of *Azolla filiculoides* was tried in the mineral solution, without any addition of organic matter extracts. This experiment was carried on for four months without the slightest deterioration in the growth and multiplication of the plants, provided light and temperature were not acting as limiting factors.

TABLE III

Shows the Average Increase in the Fresh Weight per Week in Different Illuminations and at Different Temperatures

Temperature $\pm 2^{\circ}$ C.	Per cent. increase in fresh weight per week in different light intensities			Remarks
	71.88 foot-candles	122.5 foot-candles	191.3 foot-candles	
15° C.	110.5	164.0	224.0	With the increase in temperature and light intensity there was a marked increase in growth. Illumination in all cases was for 16 hours daily.
20° C.	129.0	196.6	254.0	

DISCUSSION

1. *Table I.*—The growth of *Azolla filiculoides* was the least in the tank water. The plants showed better growth in the mineral solution as compared to the tank water.

The addition of the yeast and peat extracts definitely improved the size and growth of the plants. The addition of yeast extract proved better than peat and the addition of more quantities of yeast or peat resulted in greater growth, but not proportionately.

2. *Table II.*—When *Azolla filiculoides* was cultivated under controlled conditions in the mineral solution with and without yeast extract in low (100 watts) and high (400 watts) light intensities at $20^{\circ} \pm 2^{\circ}$ C. there was 8 to 10 per cent. more growth in the mineral solution containing yeast extract.

3. *Table III.*—*Azolla filiculoides* was successfully cultivated in the mineral solution at 15° and $20^{\circ} \pm 2^{\circ}$ C. in the low, medium and high light intensities for four months continuously. The growth, multiplication and health of the plants was very satisfactory provided light and temperature were not acting as limiting factors.

Plant Growth Substances.—In 1934 Nicol¹⁵ suggested the name 'phytamin' as a generic name for the accessory food factors. The name 'phytamin' was intended to cover the sense of Bottomley's 'auximone'. Nicol suggested that the term 'auximone' is historically valid and it ought to be restored.

Popoff¹⁷ found that extracts of the roots, stems and growing tips of seedlings of maize greatly stimulated the growth of *Euglena gracilis*.

It seems that growth-promoting hormones and 'auximone' are allied and in their presence an increase in the size of the plants and the plant cells takes place as was noted by Ashby and Tincker.¹⁸ Yeast extract is a source of 'auximone' and Boysen Jensen⁹ has produced growth-promoting substances from various fungi and bacteria. Indole-acetic acid, which is a very growth-promoting substance, is present in yeast in minute quantities, and this fact is mentioned by Nicol¹⁶ in his book. It is, however, important to remember that even the presence of 'auximone' will not produce growth unless and until the environmental conditions of light, carbon dioxide, temperature, culture solution, etc., are present in a favourable form. If the environmental conditions are favourable for the growth of the plants, then it is unnecessary to add organic matter, because growth will take place even in a purely dilute mineral solution.

SUMMARY AND CONCLUSIONS

1. *Azolla filiculoides* was grown in the green-house in pyrex glass beakers in one litre solution of quarter strength of Hoagland solution with and without yeast and peat extracts of six, three and

one gram respectively. The Hoagland solution is referred in the paper as the mineral solution.

2. The plants which were supplied with the yeast or peat extracts showed better growth and developed larger size as compared to the plants developed in the mineral solution alone.

3. The increased doses of the yeast and peat extracts induced greater growth but not proportionately.

4. Next, the plants were grown under controlled conditions of light and temperature at 15° and 20° C. in the mineral solution, with and without aquatic extract of three grams of yeast. In one week, the set with yeast extract showed 8-10 per cent. more growth.

5. After these two preliminary experiments, *Azolla filiculoides* was successfully grown in pure mineral solution for four months under controlled conditions of light and temperature.

6. The growth and the health of the plants continued to be very good, provided light and temperature were not acting as limiting factors.

7. The possibility of growing *Azolla filiculoides* in a physiologically balanced mineral solution under suitable environments of light and temperature was proved beyond doubt.

8. The addition of extracts from yeast and bacterized peat improved the growth and size of the plants, but their addition to the mineral solution was not essential for growth.

9. It is now known that 'auximone' obtained from yeast extract and some growth-promoting hormones have similar constituents of indole acetic acid and certain amino acids, which are excellent growth promoters.

10. It seems probable that *Azolla filiculoides* is capable of producing 'auximone' within itself under suitable conditions of growth. This conclusion is drawn from the fact that it has been successfully grown in pure mineral solutions.

11. The work under report was done during the author's leave from the Punjab Agricultural College, Lyallpur, at Berkeley, the University of California. He is to record his thanks to Professor A. R. Davis, for his guidance and advice in the course of this work.

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**HAPALOPHRAGMIUM PONDEROSUM SYD.
ON ACACIA LEUCOPHLOEA WILLD.**

BY M. J. THIRUMALACHAR

Department of Botany, Central College, Bangalore

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THE genus *Hapalophragmium* was first established by H. and P. Sydow in 1901 to include a species of rust on *Derris corrugata*⁶ and named it *H. derridis* H. et P. Syd. A new combination was made by Sydow⁷ by changing *Triphragmium setulosum* (Pat.) to *Hapalophragmium setulosum* (Pat.) Syd. Later on Sydow and Butler⁸ described a new species of *Hapalophragmium* on *Acacia leucophloea* which forms tumors, and named the species *Hapalophragmium ponderosum* Syd. These gall-like structures were originally mistaken to be due to insect attack by many observers. Baccarini¹ described an allied gall-forming *Hapalophragmium* on *Acacia* sp., viz., *H. acaciae*. Recently *Hapalophragmium Tandonii* Mitter has been observed on the leaves and petiole of *Acacia leucophloea* Willd. by Sydow, Mitter and Tandon⁵.

DESCRIPTION OF THE RUST

Acacia leucophloea is a thorny tree growing in dry waste lands. Numerous tumors hanging persistently at the tips of the branches are characteristic of an infected tree (Fig. 1). After one or two seasons, the tumors at the tips of the branches dry up and turn black in colour. Freshly formed tumours are of small size, with islands of green indicating new outgrowths. Tumors are formed either by infection of flower buds or very young twigs. One can observe the early stages of infection soon after the flowering season. The growth of the infected flower is very rapid due to hyperplasia, resulting in the formation of a tumor.

The first external sign of infection is the enlargement of the peduncle and the thalamus (Fig. 2), and as growth advances they become more and more globular and develop into tumors. In early stages the remnants of the flower head can be made out on the crown of the hypertrophied thalamus (Fig. 2).

In many cases the pods are seen to be borne on the swollen thalamus (Fig. 2). In such cases infection must have taken place after the pod had been formed. In some cases the pods themselves had developed tumor-like excrescences (Fig. 3).

The tumors found on the stem are few in number and less pronounced than those on the flower buds, as they seem to develop slowly and remain on the plant for a very long time because of their firm attachment to the thick trunk. On the other hand, the tumors formed by the infection of flower buds drop off following the withering of the peduncle. Young galls, 3 to 5 mm. in diameter, examined

through a magnifying lens, show tiny black specks incrustated over their surfaces.

Preliminary study of the tumors collected in different seasons were made with free-hand sections cleared in lactophenol. Material for cytological study was fixed in Bouin's fluid, and Flemming's weaker solution, embedded in paraffin after following the usual procedure. Sections of 8 to 10 μ in thickness were cut, and stained with Heidenhain's iron-alum hæmatoxylin, with orange G as counter-stain. Sections for the study of pathological anatomy of the gall were stained with safranin, with gentian violet in clove oil as counter-stain. For cytological studies, the tumor material owing to its very hard nature, was cut into thin slices, and infiltrated in glycerine after fixation to lessen the hardness. Material for spore-germination studies was collected from well-developed tumors. The technique followed for germinating the spores was as described by the writer.¹⁰

SPORE FORMS

1. *Telia*.—*Telia* develop within the tumor when they are fully developed. Sydow and Butler⁸ have given a brief description of the teleutospores. *Telia* can be made out macroscopically by their fluffy powdery appearance. The plectenchyma formed four to five cells deep in the tissue of the tumor, gives rise to slender sporophores, from the tips of which spore initials are abstricted. From the tip cell by division, three cells are cut off, which are arranged in a triradiate manner, the odd spore being terminal. With the development of the spores in the enlarged sorus, the epidermis is ruptured. The spores remain attached to the stalk persistently within the telia (Fig. 4).

The mature teliospores are three-celled. They are colourless when young, and become reddish-brown when mature, due to the accumulation of reserve materials. The germ pores are situated opposite the place of attachment (Fig. 14). They are made out in the mature spores, and become evident when the promycelia emerge as small pellicles. The spores are binucleate when young, and uninucleate when mature. The stages in the fusion of the two nuclei were observed in many cases. The nucleus after fusion possesses two nucleoli, which later on fuse.

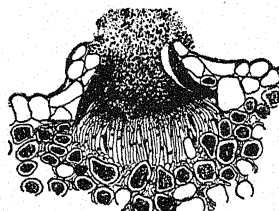
GERMINATION OF TELEUTOSPORES

The spores from telia which are freshly formed germinate readily without a period of rest being necessary (Fig. 7). Spores from very old tumors do not germinate, as their contents have deteriorated. Fresh spores germinate within 24 hours, when placed on films of water condensed on the slides following the method suggested by the author.¹⁰ Submerged spores rarely germinate, and do not develop basidiospores, but only long sterigmata. After the extrusion of the germ tube, the promycelium develops and cuts off four cells. From each of these, a sterigma is developed which abstricts off a spore at the tip (Fig. 7). The first basidiospore to be

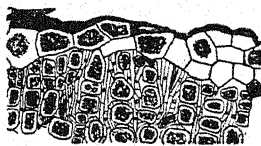
formed is the topmost one, developed from the sterigmata formed by the tapering of the tip of the promycelium. The basidiospores are uninucleate in early stages and binucleate when mature. They are oval in shape and measure $10 \times 8 \mu$ (Fig. 6). Germination of basidiospores even while still attached to the sterigmata was noticed in many cases. Development of secondary sporidia was not observed, as in *Uromyces Hobsoni*.

PYCINIA

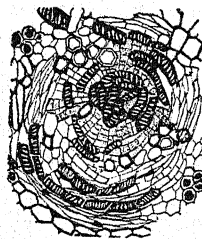
During the course of these studies the author was able to make out large number of pycnia in sections of the tumor. Sydow and Butler⁸ in describing this species do not mention the occurrence of pycnia. They are found in abundance on young tumors, and on new outgrowths from old tumors. This is the first record of pycnia for the genus *Hapalophragmium*. Pycnia are subepidermal extending from the epidermis well into the mesophyll. In sections they are oval in shape, and much flattened. The pycnosporophores are radially arranged. In a mature pycnium the ostiole is formed by the rupture of epidermis and cuticle, and contain nectar in which masses of pycnospores are embedded (Figs. 8 and 11). Pycnial initials are formed by the concentration of hyphæ beneath the epidermis. The pycnial mycelia are uninucleate (Fig. 11). Pycnospores are oval or spherical in shape. In studying pycnia in sections an interesting feature was noticed. Some of the pycnia were deep brown in colour even from the initial stages, and other hyalines. Their significance is not known.



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Figs. 11-14. Fig. 11. Camera lucida drawing of a pycnium showing the ostiole, and spores embedded in nectar ($\times 200$). Fig. 12. Camera

PATHOLOGICAL ANATOMY

The tumor caused by *Hapalophragmium ponderosum* Syd. can be classed under the category of hyperplasia described by Smith.³ The anatomical changes brought about are as follows: the vascular trace spreads out and structurally becomes a pseudostem. Xylem strands are drawn out towards the periphery (Fig. 10). In later stages one can hardly make out the region of vascular cylinder. Due to rapid cell divisions, the diffuse tumor cells multiply in number. The xylem cells become distributed promiscuously within the tissue of the tumor.

RESEMBLANCE TO CROWN-GALL

The close resemblance of the tumors caused by *Hapalophragmium ponderosum* to those by *Bacterium tumefaciens* with regards to pathological anatomy is extremely interesting. Both of them have tumors, which resemble each other in external form. The tubercle formed by the enlargement of the peduncle and the thalamus, has the pseudostem-like structure of Crown-gall on 'Paris-daisy,' reported by Erwin F. Smith.³

After the complete breakdown of the vascular cylinder, the formation of cambial cells takes place, distributed promiscuously. The function of the cambial cells in normal cases, is to elaborate out of its embryonic elements, tissues like xylem and phloem. But the cambium in the tumor shows various abnormalities in its development. The polarity of xylem and phloem no longer exists, but irregular development takes place. The parenchymatous cells are profusely developed due to active cell division.

Smith⁵ showed that the tissue elaborated by the cambium in Crown-gall, were of primitive and embryonic type. The cambial cells do not form mature xylem cells as in normal cases, but only tracheids. The parenchymatous cells also do not attain their normal size following cell division, but become reduced in size, and spindle-shaped. In the tumors developed by the infection of *Hapalophragmium* also similar types of development of tracheids, and spindle-shaped parenchymatous cells were observed (Fig. 9). In mature tumors the development of numerous tumor strands could be made out (Figs. 13 and 9). They show the same anatomical features reported by Smith⁴ in Crown-gall. The tracheids and spiral vessels are distinctly made out. Nuclear fragmentation reported by Smith³ in Crown-gall was not observed, though enlargement of the nucleus is of common occurrence.

Summing up the various cases in which the two show structural resemblance, it can be stated that they have in common large

lucida drawing of a pycnial initial ($\times 200$). Fig. 13. Tumor strand showing the characteristic tracheidal components and cambial cells ($\times 200$). Fig. 14. Camera lucida drawing of a teleutospore showing the germ pores, and nuclear fusion in one of the cells ($\times 600$).

masses of rapidly dividing cells reduced in size, and forming hyperplasia, primitive and embryonic tracheids, tumor strands and promiscuous distribution of cambial cells.

SUMMARY

1. *Hapalophragmium ponderosum* Syd. is found to infect *Acacia leucophalaea* Willd. and form characteristic tumors on the branches, flowers, and also pods.
2. Teleutospores are stipitate, three-celled and persistent.
3. Teleutospores germinate without a period of rest. Sporidia are binucleate and germinate *in situ*.
4. Pycnia have been reported for the first time.
5. Pathological anatomy has been studied. It resembles in all its anatomical and developmental features the Crown-gall of 'Paris Daisy' and of other plants caused by *Bacterium tumefaciens*.
6. As in Crown-gall, the occurrence of hyperplasia, tumour strands, tracheids and spindle-shaped parenchymatous cells have been noticed in the tumor formed by the rust.

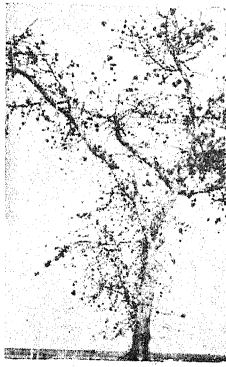
In conclusion the writer wishes to acknowledge his indebtedness to Dr. M. A. Sampathkumaran, M.A., Ph.D., Professor of Botany, Central College, Bangalore, for guidance and encouragement in the course of this work.

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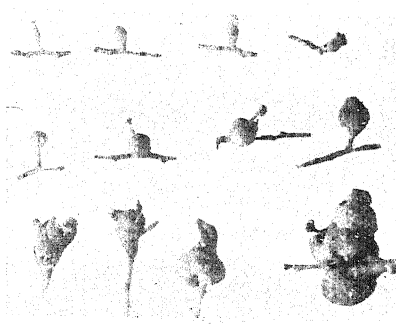
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EXPLANATION OF PLATE V

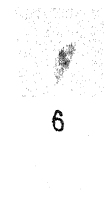
- FIG. 1. Photograph of an infected tree of *Acacia leucophloea* Willd. showing tumors at the tips of the branches.
- FIG. 2. Infected flower buds of *Acacia leucophloea* in various stages of development. The thalamus and peduncle gradually enlarge in size and form tumors. $\times \frac{1}{4}$ Nat. size.
- FIG. 3. Tumor-like excrescences on the pods. $\times \frac{1}{4}$ Nat. size.
- FIG. 4. Photomicrograph of a teleutosorus. $\times 200$.
- FIG. 5. Germination of teleutospore showing the basidiospores with short germ tubes. $\times 210$.
- FIG. 6. Photomicrograph of a binucleate basidiospore. $\times 450$.
- FIG. 7. Photomicrograph of a teleutospore showing the germination of all the three spores. $\times 150$.
- FIG. 8. Photomicrograph of pycnium. $\times 200$.
- FIG. 9. Section through an old tumor showing masses of tracheids, spindle-shaped cells and tumor strands 'T'. $\times 50$.
- FIG. 10. Section through an infected peduncle, showing the early stages in the breaking up of vascular cylinder. The xylem strands are dislodged and drawn out towards the periphery. $\times 50$.



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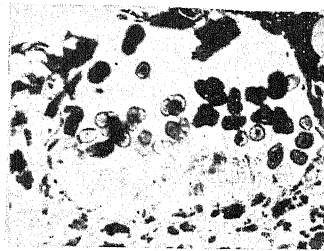
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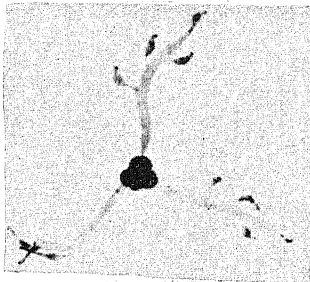
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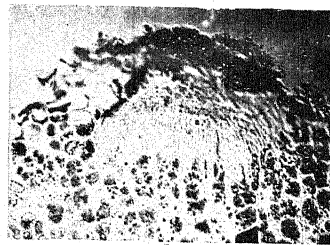
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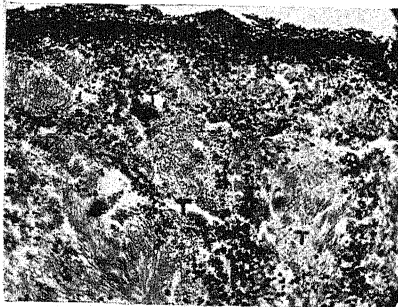
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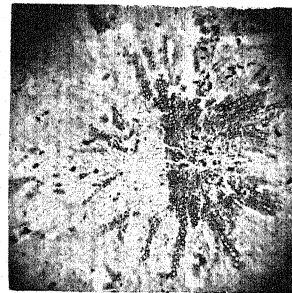
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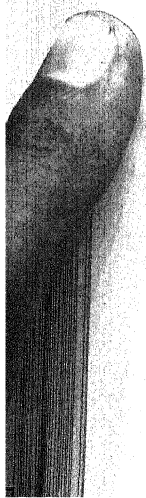


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M. J. THIRUMALACHAR—*HAPALOPHRAGMIUM PONDEROSUM*
ON *ACACIA LEUCOPHLOEA*



CONTRIBUTIONS TO THE LIFE-HISTORY OF
BIGNONIA MEGAPATOMICA

BY B. G. L. SWAMY

(Communicated by M. A. Sampathkumaran)

Received for publication on April 12, 1941

INTRODUCTION

VARIOUS members of the Bignoniaceæ are cultivated for their shade in coffee plantations in the Tropics. Our knowledge concerning the morphology of the ovules and seeds is restricted to the work of Mauritzon (1935). In that paper he has described the embryo-sac and endosperm formation in six genera. There he also gives a brief review of the work done till then on that Family. The present work was undertaken with a view to throwing more light on some of the members of the Bignoniaceæ.

MATERIALS AND METHODS

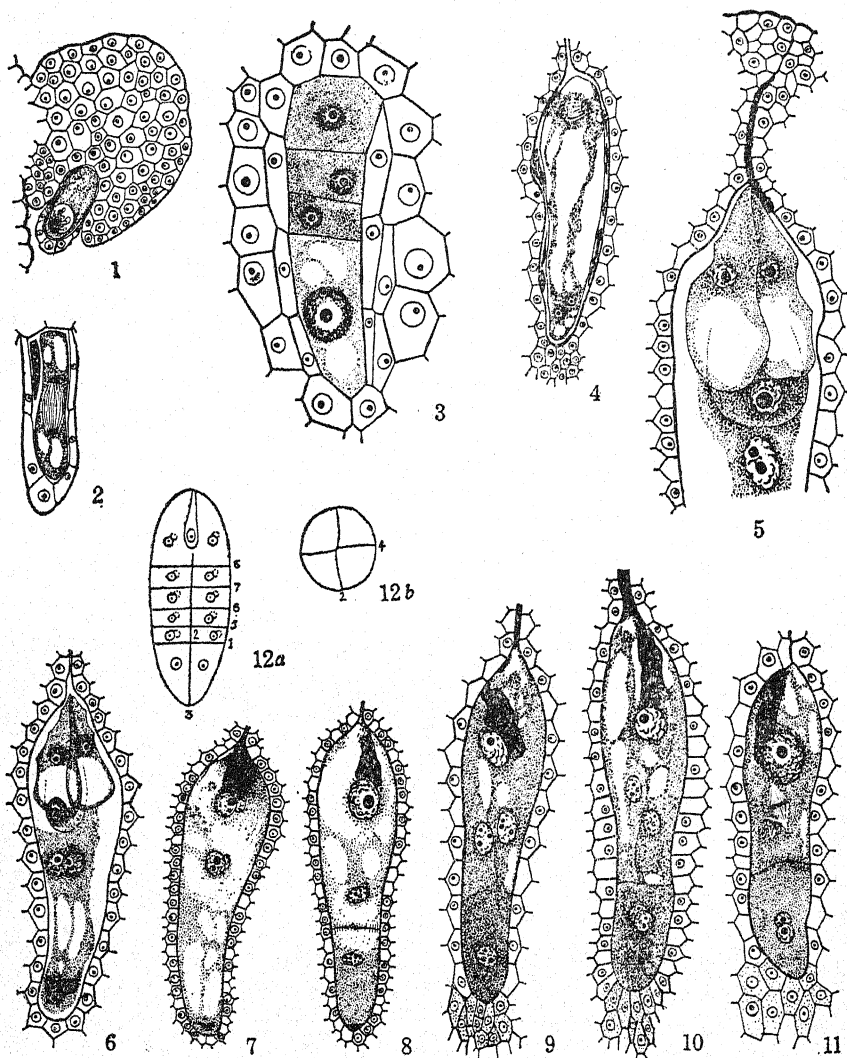
Material was fixed in Allen's modified Bouin, and sections 8 to 14 μ in thickness were cut; Heidenhain's Iron-Hamatoxylin was used for staining.

MEGASPOROGENESIS

The archesporial initial is hypodermal in origin and without cutting off any parietal layers, directly functions as the Megaspore mother cell (Fig. 1). In the very early stage the single integument differentiates around the base of the archesporial cell. When the archesporial cell is in the megaspore mother cell stage the integument consists of two to three layers of cells in thickness; at the tetrad stage four to six layers; and at the eight-nucleate embryo-sac stage eight to ten layers of cells. A linear tetrad (Fig. 3) is formed following the two reductional divisions. Subsequently the chalazal megaspore enlarges and develops into the embryo-sac according to the normal-type. Following the enlargement of the chalazal megaspore the surrounding nucellar cells disorganise and contribute food materials.

The nucleus in the chalazal megaspore undergoes the usual divisions (Fig. 4) and develops into the eight-nucleate embryo-sac (Fig. 6). At maturity, the embryo-sac occupies a wide space after encroaching upon the nucellar tissue which becomes disorganised.

The egg apparatus is organised at the micropylar end. The synergids enlarge in size and show the usual basal vacuoles (Fig. 5). The synergids in all cases observed were hooked. Similar instances have been reported by Mauritzon in *Catalpa* (1935), in *Lythraceæ*



Figs. 1-12b.—Fig. 1. Megaspore mother cell. $\times 400$. Fig. 2. Division (reduction) of the megaspore mother cell. $\times 600$. Fig. 3. Linear tetrad of megaspores, the lowermost megaspore enlarging. $\times 900$. Fig. 4. Four-nucleate embryo-sac. $\times 450$. Fig. 5. Micropylar half of the mature embryo-sac and the pollen tube in the micropyle. $\times 900$. Fig. 6. Mature eight-nucleate embryo-sac. $\times 450$. Fig. 7. Double Fertilization. $\times 450$. Fig. 8. First division of the endosperm nucleus. $\times 450$. Figs. 9-11 and Fig. 14. Stages in the development of the endosperm, chalazal haustorium and endosperm. $\times 450$. Figs 12a and 12b. Diagrammatic representation of the sequence of walls laid down during the successive divisions of the primary endosperm nucleus.

by Joshi and Venkateshwaralu (1935-36), and in Melastomaceæ by Subramanyam (unpublished).

The polar nuclei fuse just before fertilization. The antipodals begin to degenerate when the embryo-sac is mature and in majority of cases disappear after fertilization.

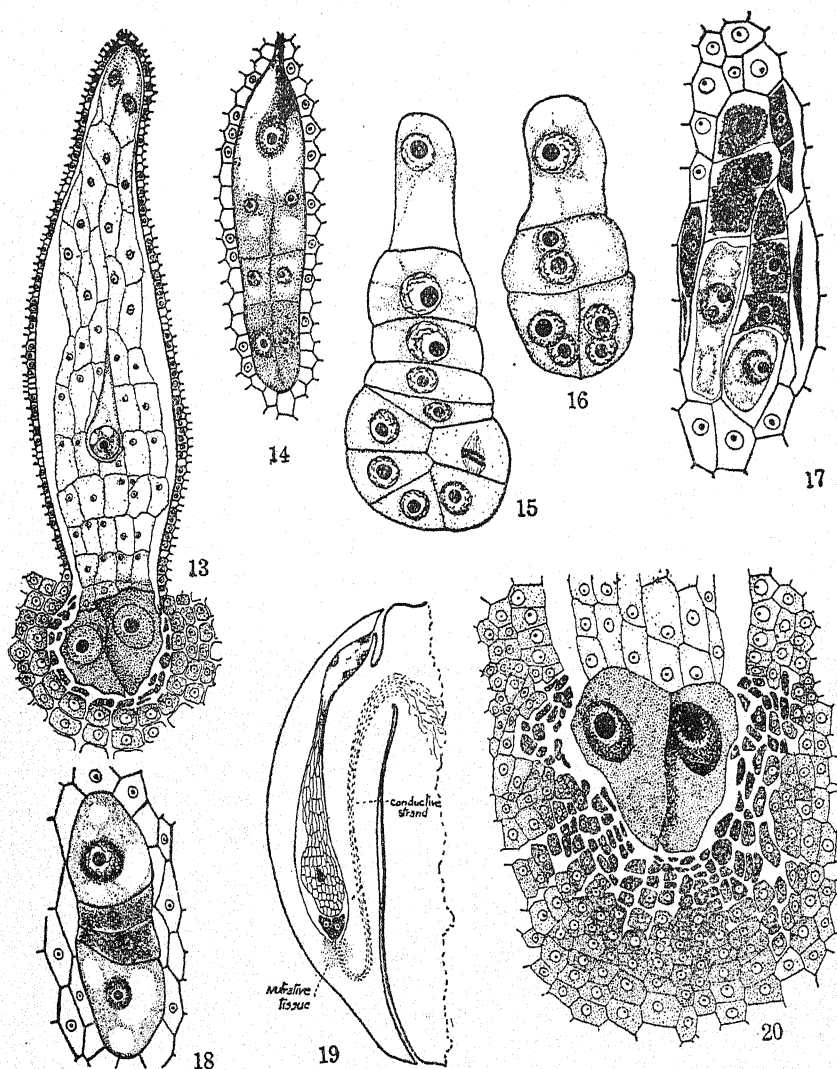
The entry of the pollen tube into the embryo-sac through the micropyle was observed in many cases (Fig. 5). The pollen tube crushes the synergids in its passage. Double fertilization was made out in many instances (Fig. 7).

ENDOSPERM

The primary endosperm nucleus divides and forms two nuclei, followed by wall formation, which separates a basal chalazal chamber and an upper micropylar chamber (Fig. 8). The nucleus of the micropylar chamber divides by a vertical wall and again by another vertical wall at right angles to the previous one, so that a single tier of four cells will be organised just above the chalazal chamber (Figs. 9, 12*a* and 12*b*). At about the same time the nucleus of the chalazal chamber also divides by a vertical wall into two cells (Fig. 10). These do not divide further and constitute the endosperm chalazal haustorium, whence it begins to eat up the surrounding nucellus.

The nuclei of the single tier of four cells in the micropylar chamber undergo division by transverse wall, resulting in two tiers of four cells each (Figs. 11 and 14). Of these two the lower tier remains without division for a time, only the upper tier dividing likewise and contributing new endosperm cells which gradually become elongated and larger in their respective sizes and thinner in their contents (Figs. 13 and 19). The divisions come to a stop after twenty to twenty-five tiers of cells are formed. In the division of the four nuclei of the last tier of cells which is towards the extreme end of the micropyle the vertical walls do not develop and all the four nuclei will be coenocytic (Fig. 19). This coenocytic cell gradually enlarges, its protoplasm becomes dense and the nuclei become hypertrophied which ultimately become haustorial. The enlargement of the cell is associated with the disintegration of surrounding nucellar layers of cells. At this stage vertical and oblique walls may appear in the older endosperm cells and thus cause a further increase in the number of endosperm cells. The sequence of the divisions of the endosperm nucleus is graphically represented in Figs. 12*a* and 12*b*.

The nucellar tissue in contact with the chalazal haustorial cells disorganise and contribute food materials (Figs. 13 and 20). The nucellar cells, three to five layers, below the haustorium arrange themselves in regular rows in which the protoplasm is very conspicuous. Such radiating arrangement of cells (Fig. 20) at the chalaza have been reported in some members of Scrophulariaceæ (Krishna Iyengar, 1940) and in *Enalus* (Kausik, 1940). These nucellar



Figs. 13-20.—Fig. 13. Endosperm chalazal haustorium and endosperm in which the zygote is resting. $\times 200$. Fig. 14. A stage resulting from the one shown in Fig. 11. $\times 450$. Figs. 15 and 16. Stages in the development of the embryo. $\times 900$. Fig. 17. Double tetrad. $\times 900$. Fig. 18. An abnormal tetrad in which two megaspores are enlarging. $\times 450$. Fig. 19. Diagram to illustrate the four regions of the adult endosperm tissue, the nutritive tissue and the conductive strand. Fig. 20. Chalazal haustorium showing the disintegration and the radiating arrangement of the surrounding nucellar cells. $\times 400$.

cells probably help in the conduction of food materials to the embryo-sac.

It is also interesting to note that a strand of cells with dense protoplasm connects the vascular traces and the radiating cells at the chalaza (Fig. 19). Judging from their appearance and position it is safe to conclude that these too take part in the conduction of food materials.

The endosperm, after sometime becomes a longitudinally stretched body in which the size of the cell extends from chalaza to micropyle (Figs. 13 and 19). The mature endosperm tissue is composed of a large number of cells of different sizes. Four distinct regions can be observed (Fig. 19) depending upon the size and compactness of the cells :—

1. Two cells with conspicuous protoplasm and being haustorial in function at the chalazal end,
2. A mass of cells towards the chalaza arranged compactly and small in size and containing dense protoplasm,
3. Placed above that, a tissue with large sized cells and loosely arranged with cell contents not staining deeply, and
4. A coenocytic cell which is haustorial consisting of four nuclei.

It is important to note that Mauritzon in his treatise on the Bignoniaceæ admits that he did not observe the older stages of some genera (*Pithecoctenium*, *Bignonia*, *Jacaranda*) as regards the fate of the late endosperm and its position towards the micropyle. He assumes that the older stages of the above genera may correspond to those that he observed in *Catalpa* and that to regard this hypothesis correct a more detailed story is necessary. He further remarks in general to those species in which *Catalpa*-type of endosperm formation prevails, that : regarding the eventual appearance the outermost micropylar endosperm portion, can with uncertainty (1) remain prominent, (2) the four micropylar cells can, particularly after their contribution by abstriction of cells below, become four secondary micropylar haustoria, (3) or they can assume the same appearance as their last daughter cells and can also form the last, upper, large endosperm cells, whose position and form can, as in other endosperm cells, become altered by growth and displacement. It is not clear if the above alternatives are confined to any one particular species or to all the species in which the *Catalpa*-type of endosperm formation prevails.

In the present investigation it was observed that the last tier of cells towards the micropyle is coenocytic with four enlarged nuclei and taking up a haustorial function.

The fertilised egg rests for some time without any division. The remnants of the pollen tube persist for a long time before degeneration. When the endosperm is about sixty to seventy cells, the fertilized egg migrates to the centre and can be made out among

the cells of the endosperm (Fig. 13). Here the zygote undergoes its first division which is transverse, and the subsequent development conforms to the *Capsella*-type (Figs. 15 and 16). The suspensor cells are very conspicuous and vary from five to six cells in number.

CONCLUSIONS

The outstanding feature in the study of the morphology of the Bignoniaceæ is the development of endosperm tissue. Mauritzon after a detailed study of various members of Bignoniaceæ, differentiated two main types of endosperm formation, (1) the *Catalpa*-type and (2) *Incarvillea*-type. *Bignonia megapotamica* belongs to the former type as regards the endosperm development with minor variations. The chalazal haustorium is composed of only two cells and not four, as observed in the many members of Bignoniaceæ by Mauritzon. The occurrence of a coenocytic micropylar haustorial cell in *Bignonia megapotamica* is an interesting feature.

The radiating cells at the chalaza, and the strands of cells connecting the vascular bundles and chalazal region might be helpful in the conduction of food materials as already stated in the earlier part of this paper.

The occurrence of double embryo-sacs in the nature of double tetrads (Fig. 17), etc., have been made out in this plant. The double embryo-sacs are frequently reported in a number of plants, but the uncertainty remains as to whether both of them are functional normally. The chalazal megaspore in the linear tetrad is functional in normal places but frequently instances were met with, wherein the micropylar megaspore in addition to the chalazal one enlarged (Fig. 18.)

SUMMARY

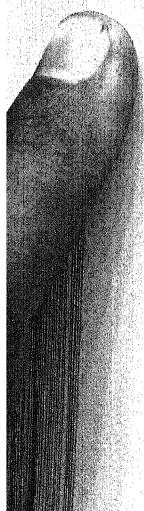
1. There is a single hypodermal archesporium, which functions directly as the megaspore mother cell.
2. The embryo-sac is of the monosporic eight-nucleate type.
3. Double fertilization has been observed.
4. The endosperm formation corresponds to the *Catalpa*-type reported by Mauritzon, with this difference that only two cells are present in the chalazal haustorium, and the cell at the micropylar end is coenocytic, being quadrinucleate. It is haustorial in function.
5. The development of the embryo is of the *Capsella*-type.

ACKNOWLEDGEMENTS

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CYTOGENETICAL STUDIES IN NICOTIANA

Part I. Cytology of *Nicotiana glutinosa* and *N. Tabacum*
var. *macrophylla* and the F_1 hybrid between them

By T. S. RAGHAVAN, M.A., PH.D. (LOND.), F.L.S.

AND

A. R. SRINIVASAN, M.Sc.

Annamalai University

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I. INTRODUCTION

THE family *Solanaceae* includes genera which lend themselves easily to cytological and cytogenetical analysis and show many interesting meiotic features which are made use of to elucidate many a puzzling problem in the realm of Cytogenetics. *Datura*, species of *Solanum*, *Petunia*, *Lycopersicum* and other genera have been worked out in great detail and they have yielded very valuable results.

The genus *Nicotiana*, which includes about 50 species and which are grouped under four heads *Tabacum*, *rustica*, *petunioides* and *polidiclia* (Hector, 1936) has been for a long time the favourite of cytogeneticists. The work in this genus has been many sided. The genus affords material for anatomical, morphological, physiological and cytological studies. But most of the important work has been in the cytogenetical field. New avenues in this plant research were thrown open after the effect of X-rays and alkaloids like Colchicine, *Acenaphthene*, etc., on the reproductive cells of the plants was discovered. These upset the mitotic or mitotic balance of the plant and these aberrations throw light on polyploidy, sterility, etc.

Genetical work was started because *Nicotiana* was important as an agricultural product. The genus includes many varieties of two species, *rustica* and *Tabacum*, widely cultivated throughout the world, for their leaves which are used for manifold purposes under the popular name of tobacco. Because of its economic importance, genetic experiments were carried on in the agricultural research

departments where a synthesis of the various desirable qualities in different varieties, was attempted. The interest was further promoted by the success that this breeding work promised.

The genetical work in *Nicotiana* can be traced back to very early times. It was Comes (1899) and Anastasia (1906) who, on the basis of the results got in genetic experiments, referred the commercial varieties of *Nicotiana*, as derivatives from a limited number of fundamental forms. In order to find out which form was to be held as fundamental, intervarietal crosses were made by Setchell in 1909 (Setchell *et al.*, 1921). Other studies, purely genetical, were made by Allard (1919) who studied the inheritance of flower colour in *Nicotiana sylvestris* and *N. Tabacum*.

The classification and description of all the Indian varieties of *Nicotiana Tabacum* and *N. rustica*, was published by Howard and Howard (1910). Since then breeding work in tobacco has been vigorously carried on in many agricultural research stations. A quantitative study on the inheritance of the desirable qualities in various varieties, is made in order to enable the breeder to understand how to bring together and fix desirable characters in one progeny. Such quantitative genetical studies through intervarietal crosses have been made by Goodspeed (1912, 1913a).

Cytological studies have been extensively done in *Nicotiana* though no such work has been done in any of the Indian varieties. White in 1913 found the haploid chromosome number of *N. Tabacum* to be 24. It was confirmed later by Palm (1922), Goodspeed (1923) and Rybin (1927). Goodspeed in 1923 gave a general account of the cytology of the species of *Nicotiana*. The same author in 1924 published a list of chromosome numbers of 13 species of *Nicotiana*. Of these, three species had $n=9$, 6 species showed $n=12$ and 4 species had $n=24$. Clausen and Goodspeed (1926a) reported the spontaneous occurrence of a fluted form in *N. Tabacum* cultures, which on cytological analysis was found to be a monosomic ($2n-1$). Studies of transmission of the character fluted were verified cytologically by finding out the percentage of functioning gametes having $n-1$ chromosomes. A haploid plant of *Nicotiana Tabacum* variety *purpurea* was got from the cultures of *N. sylvestris* \times *Tabacum* hybrids, whose origin is ascribed to the parthenogenetic development of the egg of the *Tabacum* parent (Chipmann and Goodspeed, 1927). No pairing was observed at diakinesis and previous stages and a true metaphase was found lacking. All the chromosomes did not arrange themselves in the equatorial plate. Dyads and viable pollen grains with the full haploid chromosome set of *Tabacum* were produced. The replacement of homotypic division in the place of heterotypic division was suggested as the possible cause. The results from the study of this haploid have been very helpful to elucidate the modes of pairing in species hybrids with *Tabacum* as a parent.

Goodspeed (1933) gives a list of chromosome numbers of 40 species of *Nicotiana* in which he has reported haploids in four species,

glutinosa, *Tabacum*, *Longsdorffi* and *nudicaulis*. Three amphidiploids, *digluta*, *diglutosa* and *disuaveoli* and aneuploids are made mention of. The numbers characteristic of these 40 species are 9, 10, 12, 16, 20, 22, 24 and 32. A grouping of these species into distinct classes on the basis of chromosome numbers and other morphological features is also given. The cytology of a triploid *N. tabacum* has been studied by East (1933).

The real importance of this genus lies in the extensive studies made on the large number of interspecific hybrids in this genus and their progenies. These are all the more interesting and easy because of the ease with which the species are crossed with each other. The compatibility that exists between the various species and the gradation of sterility in these hybrids are well corroborated by the interesting features of meiosis. The peculiarities met with in this case give valuable clues to the proper understanding of the meiotic mechanism of other interspecific hybrids.

A study of the history of interspecific hybridisation in this genus leads us to the middle of the 19th century. Reference to interspecific hybrids in *Nicotiana* during 1849 and 1881 is made by Clausen and Goodspeed (1925). Gärtner (1849) and Focke (1881) have described hybrids between *N. Tabacum* and *N. glutinosa* and have studied purely genetical problems as the variation of hybrid vigour with the use of different varieties of *Tabacum* in the species crosses.

Interspecific crosses were made in 1910 by Setchell between *N. Tabacum* and *N. sylvestris*. In 1911 they were backcrossed to the parents and the partial sterility that was exhibited by these hybrids was described (Goodspeed, 1913). An abscission layer is said to be formed at the end of the pedicel, which causes the flower to fall down even before fertilisation in some cases. Goodspeed and Ayres (1916) during the study of partial fertility of the above said hybrids, tried to inhibit the abscission of fruits and flowers, in which only a few or none of the ovules have been successfully fertilised. In another paper Goodspeed and Kendall (1916) described the mode of abscission of flowers and fruits and evidences were given as to the relation between the successful pollination and fertilisation on the one hand, and abscission of flowers and fruits on the other. Goodspeed and Clausen in 1917 described several F_1 hybrids made with *N. sylvestris* and several varieties of *N. Tabacum* as the parents. A general description of these hybrids and their figures were given in that work.

East (1921) showed that by selfing partially fertile *paniculata* \times *rustica* hybrids, stable *rustica* derivatives differing from the *rustica* parent in some features could be obtained. Among the progenies some were equivalent to the *paniculata* parent also.

Goodspeed (1923) published a preliminary account of the cytology of *Nicotiana* species and hybrids. There he made mention of three crosses. They were *N. sylvestris* \times *N. Tabacum*; *N. glutinosa* \times *N. Tabacum* and *N. Tabacum* \times *N. paniculata*. Of these three a

short account of the cytology of *Tabacum* × *sylvestris* hybrid was given. The $12_{II} + 12_I$ configuration, typical of the Drosera scheme of chromosome pairing, and the random distribution of univalents, without any of the univalents being left in the plasma, was met with in that case. Abnormal tetrads were occasionally found.

The occurrence of a tetraploid *glutinosa* × *Tabacum* hybrid was reported by Clausen and Goodspeed (1925). The F_1 hybrids were sterile and only one was found to be partially fertile. From this fertile hybrid an F_2 generation which was comparatively more fertile was raised. Among the plants belonging to the F_2 generation, the tetraploid was distinguished by its stalwart growth and extreme fertility. Cytologically this exhibited regular meiosis showing 36 bivalents at MI and good, healthy and viable pollen grains were formed. The method of origin of this tetraploid was not given by the author.

The cytological features of the *paniculata* × *rustica* hybrids and their derivatives were described in a subsequent paper by Goodspeed, Clausen and Chipmann (1926). In this paper East's conclusion (1921) regarding the genetic characteristics of these hybrids was discussed in the light of the cytology of the F_1 *rustica* × *paniculata* and its backcross progenies to their parents. The F_1 showed $12_{II} + 12_I$ configuration and was partially fertile. By backcrossing to *paniculata*, derivatives showing $12_{II} + n_I$ where n varied from 2 to 9, were obtained. In some cases the $12_{II} + 12_I$ configuration was met with, where division of univalents was presumed. The *rustica* backcross derivatives showed $18_{II} + 6_I$ and $20_{II} + 4_I$, but cases showing 24 bivalents were never met with. This indicates that, complete pairing of the parent *rustica* chromosomes, with the *rustica* chromosomes in the hybrid, to the exclusion of *paniculata* chromosomes, was not met with. In East's work *rustica* derivatives slightly different from *rustica* parent were obtained. These differences could be accounted for, by assuming that some of the *paniculata* chromosomes homologous to those of *rustica* have been substituted for some *rustica* chromosomes. The *paniculata* chromosomes and the *rustica* chromosomes, though homologous, are not of identical morphology and such an inclusion of *paniculata* chromosomes in the chromosome complement of *rustica* will naturally alter the morphological features of the *rustica* derivatives to some extent.

Clausen and Goodspeed (1926b) got a corrugated derivative from the backcross of *Nicotiana Tabacum* var., *Macrophylla* × *sylvestris* with the *Tabacum* parent. This was found to be the monosomic $2n-1$.

Complete sterility was met with in the case of the hybrids of *N. bigelovii* ($n=24$) with *N. suaveolens* ($n=16$) and with *N. glutinosa* ($n=12$) (Goodspeed and Clausen, 1927 a) and in both the cases complete asynapsis was recorded. Formation of multipolar spindle was observed in the *glutinosa* × *bigelovii* hybrids. The irregular random distribution of chromosomes resulted in the formation of many

microcytes, and micronuclei in the tetrads. Formation of dyads owing to the non-disjunction at M_1 and to the subsequent normal homotypic division was also recorded.

Analogous in all its meiotic features to *rustica-paniculata* hybrid and exhibiting the drosera scheme of pairing was the hybrid *Tabacum* \times *sylvestris* (Goodspeed and Clausen, 1927 b). Through back crosses with the parents, derivatives slightly different from *Tabacum* and *sylvestris* respectively, were obtained.

The occurrence of a fertile amphidiploid derivatives from *N. Tabacum* \times *N. glutinosa* hybrid which functioned like a normal species having 72 chromosomes 48 of *Tabacum* and 24 of *glutinosa*, was reported by Clausen (1928). This amphidiploid was named as *digluta*. On back-crossing this with the parents a 48 chromosome derivative in the case of *glutinosa* and a 60 chromosome derivative in the case of *Tabacum* were got.

The origin of *Tabacum* from an amphidiploid of *N. tomentosa* ($n = 12$) \times *N. sylvestris* ($n = 12$) was proved by Goodspeed and Clausen (1928), through a comparative study of the three hybrids *N. Tabacum* \times *N. sylvestris*; *N. sylvestris* \times *N. tomentosa* and *N. tomentosa* \times *N. Tabacum*. Both the hybrids exhibited the Drosera scheme of pairing (*Tabacum* \times *sylvestris* and *Tabacum* \times *tomentosa* hybrids). The absence of pairing within the haploid set of chromosomes having been previously proved, the pairing should be between 12 chromosomes of *Tabacum* with 12 of *sylvestris* in one hybrid and with 12 of *tomentosa* in the other. So it follows that out of 24 chromosomes belonging to the haploid set of *Tabacum*, 12 are homologous with the 12 chromosomes of *tomentosa* and the other 12 are homologous with 12 chromosomes of *sylvestris*. So if a hybrid is got between *tomentosa* and *sylvestris* it should resemble *Nicotiana Tabacum* as it contains a chromosome set completely homologous with the haploid chromosome complement of *Tabacum*. This cross was effected and a sterile hybrid showing complete asynapsis was got which, except for some differences, resembled the *Tabacum* plant.

From the above facts *Tabacum* was presumed to have been derived from a fertile amphidiploid of *tomentosa* \times *sylvestris* hybrid. Because of the differences in morphological features between the hybrid and *Tabacum*, the amphidiploid could not be taken as the immediate progenitor of *Tabacum*. The chromosomes of the amphidiploid have undergone some structural changes and have become stabilised as the chromosome set of *N. Tabacum*. Thus from cytological data the origin of *Tabacum* could be established.

Lammerts in 1929 described the cytology of the back-cross derivatives of *paniculata* \times *rustica* hybrids. The same author in 1932 suggested a general method of origin of higher chromosome numbers other than the number found in the polyploid series. This is by selfing hybrids which are diploid for one species and haploids for the other. This suggestion is made after a study of a 30_{II} derivative got by the union of an unreduced egg of *rustica* \times *paniculata*

hybrid with a haploid gamete of *paniculata*. From this, by constant selfing, derivatives having 56, 58, 59 and 60 chromosomes were got and all of these were fertile. He also discussed certain difficulties met with in describing the origin of higher chromosome numbers by way of species crosses.

Webber (1930) got a sesquidiploid having 60 chromosomes that arose from the union of a diploid *Tabacum* egg with 48 chromosomes with a haploid sperm of *sylvestris* having 12 chromosomes.

The phylesis in the genus *Nicotiana* has been discussed in the light of chromosome number, chromosome morphology, modes of pairing and formation of chiasmata in the individual species by Goodspeed (1934). The behaviour of interspecific hybrids between the species under consideration and cytological features of amphidiploids that have arisen out of some of these hybrids, are discussed as further evidence in support of this opinion regarding the evolution species in the genus.

Müntzing (1935) in his paper about the chromosome behaviour of *nicotiana* hybrids, describes the cytological features of the hybrid between *N. bonariensis* ($n = 9$) \times *N. Longsdorffi* ($n = 9$). Ordinarily when both the parents have the same number of chromosomes, the interspecific hybrid between them has been seen to show complete or partial synapsis or complete asynapsis. In this case, however, a considerable amount of trivalent formation was met with along with bivalents and univalents. Chromatin bridges were observed in anaphases indicating structural changes of chromosomes during meiosis.

In the studies of *N. Tabacum* \times *glutinosa* hybrids mentioned above, much attention was not devoted to the cytology of F_1 hybrid. The first detailed study of the meiosis of F_1 hybrid was made by Munting (1935) who reports very weak pairing among the 36 chromosomes of the F_1 hybrid. Random distribution occurs and no unreduced gametes are formed according to him. In this paper a detailed account of the cytological investigation of the parents and the F_1 hybrids between a South Indian chewing variety of *Tabacum* and *N. glutinosa* is described.

II. CYTOLOGICAL TECHNIQUE

Seeds of parents were obtained from Coimbatore and the crosses were done in the Botanical Gardens, Annamalainagar. Description of the parents and details of the technique of crossing have been given elsewhere (Raghavan and Srinivasan, A. R., 1941). Flowers of the hybrid and the parents were ready in July for cytological investigations.

Root tips of the hybrid and the parents could easily be obtained from the plants grown in pots and they were fixed in various fixing fluids. Maximum mitotic activity was observed from 1-30 P.M. to 3-30 P.M. and root tips were fixed at this time. The fixatives used

were Karpechenko's modification of Navaschin's chromacetic formalin, Allen's Bouin's fluid, Navaschin's fluid with previous Carnoy fixation and Craf's fluid. Of these Navaschin's fluid without pre-fixation with Carnoy was found to be the best.

In the case of the Navaschin's fixative, the various grades from 10% to 50% alcohol were found to be unnecessary. After preliminary washing in water the material was directly taken to 50% alcohol, dehydrated, and infiltrated with paraffin wax using chloroform as the paraffin solvent. The materials were then imbedded in soft paraffin wax of melting point 52° Centigrade.

Dioxon was tried as both the dehydrating agent and paraffin solvent and was found to yield good results though it gave some difficulty during staining with gentian-violet. Sections were cut at thicknesses varying from 12 to 15 microns and were stained both in Newton's-iodine gentian-violet and Haidenhein's iron-alum Hæmatoxylin.

Smears of anthers were also tried with good results using Belling's Navaschin fluid as the fixative. These were stained both in gentian violet and Hæmatoxylin. The correct stages for fixation in anthers were determined after examination in Acetocarmine preparations.

III. CYTOLOGY OF THE PARENTS

Though the cytology of the parent species has been described in some detail by various authors, an understanding of the cytological features of the parents that were used in this cross was found to be desirable for purposes of comparing the meiotic behaviour of the hybrid with that of the parents.

The Cytology of Nicotiana glutinosa

Somatic chromosomes.—Fig. 1 represents a somatic metaphase plate of *N. glutinosa* showing 24 chromosomes. The chromosomes on the whole are comparatively long. Goodspeed (1934) has classified the somatic complement of *glutinosa* into two classes on the basis of the length of the chromosomes. There are altogether 7 pairs of long chromosomes of which 4 pairs have median insertion region. Of the other three pairs, two have sub-median spindle attachment regions and the remaining one pair shows sub-median insertion regions, along with proximal satellites. The smaller chromosomes which are 10 in number form the second group and they are said to possess median and sub-median insertion regions. The somatic plate in Fig. 1 shows the difference in length of the chromosomes and satellites are seen in two of the long chromosomes.

Meiosis

Diakinesis (Fig. 2).—At diakinesis the 24 chromosomes are seen to form 12 pairs. Bivalents were essentially of the rod type and the ring type. The total number of chiasmata has been reported to be 22 and of the bivalents, all except 2 have two terminal or nearly terminal chiasmata (Goodspeed, 1934). Fig. 2 shows

ring bivalents and 6 rod-bivalents and another bivalent body resembling an open ring with single terminal chiasma. This probably results from terminalisation of an interstitial chiasma formed in the earlier diakinesis stages. All the ring bivalents have terminal chiasmata while the number of chiasmata in the rod bivalents could not be found out. Goodspeed (1934) reports that one interstitial chiasma is formed by the long chromosomes which have the sub-median insertion region. The bivalent which resembles an open ring is probably formed by the synapsis of two such long chromosomes, for, as Goodspeed says "in the case of the large chromosomes with a sub-median insertion region, frequently a chiasma forms in the longer arm only". If such a chiasma is formed and gets terminalised in the late diakinesis stage, which Fig. 2 represents, we can account for the formation of the open ring bivalent.

At diakinesis the bivalents are distributed on the periphery of the nucleus. All the pairs are dispersed at equal distances from each other in earlier diakinesis stages. In Fig. 2 some of the bivalents are seen to have come closer to each other. This according to Lawrence (1931) is due to a repulsion phase which begins at early diakinesis and continues until mid-diakinesis. Probably the force of repulsion between chromosomes weakens, with the inception of prometaphase.

Prometaphase.—In this stage the bivalents which have undergone maximum contraction come very close to each other and present a clumped appearance, so that individual bivalents could be distinguished only with much difficulty (Fig. 3). This stage lasts for a very short period and its inception is denoted by the disappearance of the nuclear membrane and the migration of the bivalents to the centre of the nuclear cavity. The stage comes to an end when spindle fibres appear.

Metaphase.—During metaphase the bivalents which have separated from each other are arranged in the equatorial plate. This separation is probably due to the inter-bivalent repulsion which reaches its minimum at pro-metaphase and increases with the inception of metaphase. Due to the repulsive force, the bivalents spread themselves in the equatorial region. All the 12 bivalents are seen in the same plane at metaphase I (Fig. 4).

Anaphase.—Anaphase in this case is normal. The bivalents disjoin regularly and the chromosomes reach the poles without presenting any abnormal features as bridge formation, fragmentation, etc. Some of the bivalents disjoin much later than others but the disjoined chromosomes succeed in reaching the poles. Fig. 5 shows two such bivalents and in Fig. 6 one bivalent is seen to disjoin after all the other chromosomes have reached the poles. Fig. 7 shows a normal case where all the chromosomes which have disjoined simultaneously are seen to travel up the spindle to the poles.

Interphase.—Interphase is a clearly defined stage in the case of this plant and at each pole 12 chromosomes resulting from the disjunction of bivalents at A I organise themselves into two nuclei. The nucleoli make their appearance and the chromosomes are more or less uniformly spaced (Fig. 8). This uniform distribution was recorded by Gates (1909) in *Eriogonum* and by Raghavan (1938) in *Gynandropsis*. Raghavan and Srinivasan, V. K., have found it in *Angelonia* also (1940).

Metaphase II.—The interphase nuclei lose their nuclear membrane and enter into the second metaphase stages. At this stage 12 chromosomes are seen in each of the two poles (Fig. 9). After the division of chromosomes the daughter-chromosomes reach the poles in the two spindles of A II (Fig. 10). Thus there are two spindles at the early second telophase stage.

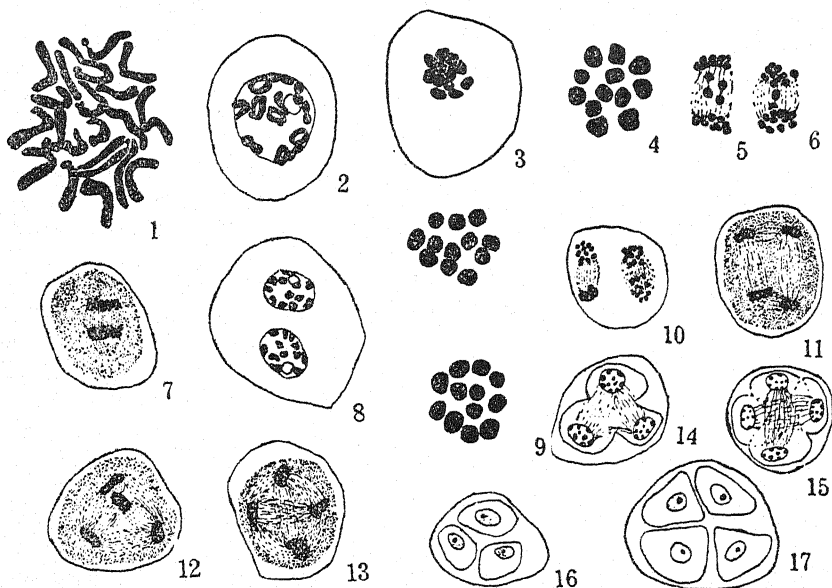
Shortly afterwards, however, two more spindles connecting the two telophase daughter-nuclei at the same pole, are formed in addition to those formed at A II (Fig. 11). Afterwards, two more spindles arise and each of the telophase group becomes connected with every one of the other three groups (Figs. 12 and 13). The formation of 6 connecting spindles is the usual case in angiosperms exhibiting the "furrow method" of pollen organisation, though examples can be given of plants differing from this. Wodehouse (1931) reports only four connecting spindles in the tetrads of *Dahlia*. In *Magnolia* the six spindles are not as distinct as in *Nicotiana* and Farr (1918) says that it is doubtful whether these should be considered as six separate spindles, or two spindles and a compound spindle.

It is not possible to find any explanation of how the two or four additional spindles arise. Wodehouse (1931) says "the work of Devise (1921) on the achromatic figure of the sporocytes of *Larix* offers some suggestion that they may be regarded as the reappearance of the heterotypic spindles split longitudinally". From this it follows that the halves of the heterotypic spindle persist intact, though in a less conspicuous form, throughout the second division stages, but in *Dahlia* the orientation of A II spindles could be in any direction making it "apparently impossible for the heterotypic spindle to persist in any tangible form through the homotypic division". So the origin of these additional spindles remains still unexplained.

When the six spindles are formed the four daughter-nuclei are orientated in two ways. In the one case all the four nuclei lie in the same plane (Fig. 13). In the other case (Fig. 12) one group of telophase is at a different focus from the other three. The former leads to the formation of an isobilateral tetrad while the latter will give rise to the tetrahedral type and exhibits a tendency towards the least surface configuration (Fig. 16). The four nuclei are approximately equidistant from each other.

Soon after the appearance of the four additional spindles in the tetrad, quadri-partition begins. Farr (1916) has described this to be

accomplished by a process of simultaneous furrowing in *Nicotiana*. In the present case the furrowing and other later stages may take place in two ways. For the tetrahedral type the furrows are formed on the periphery of the cytoplasm and extend towards the centre (Fig. 14). The furrows are wide in the present case. They have



Figs. 1-17.—*Nicotiana glutinosa*. Fig. 1. Somatic metaphase of *N. glutinosa* showing two chromosomes with satellites. $\times 3,900$. Fig. 2. Diakinesis, 5 ring bivalents are shown. $\times 2,200$. Fig. 3. Prometaphase. $\times 2,200$. Fig. 4. Metaphase I polar view showing 12 bivalents. $\times 3,900$. Figs. 5-7. Anaphase I. $\times 1,500$. Fig. 5. Two bivalents undergoing late disjunction. Fig. 6. One bivalent disjoining later than all the rest. Fig. 8. Interphase showing 12 daughter-chromosomes and one nucleolus in each of the two nuclei. $\times 2,200$. Fig. 9. Metaphase II polar view. $\times 3,900$. Fig. 10. Anaphase II showing two spindles arranged parallel to each other. $\times 1,500$. Fig. 11. Telophase II the appearance of two new spindles connecting the two telophase groups in the same pole. $\times 1,500$. Fig. 12. Tetrahedral 4 nucleate cell with 6 spindles. $\times 1,500$. Fig. 13. Isobilateral 4 nucleate cell with 6 spindles. $\times 1,500$. Fig. 14. Tetrahedral tetrad showing furrowing. $\times 1,500$. Fig. 15. Isobilateral tetrad showing the two dumb-bell-shaped bodies each having a spindle. $\times 1,500$. Fig. 16. Tetrahedral tetrad. $\times 1,200$. Fig. 17. Isobilateral tetrad. $\times 1,500$.

been found to be very narrow in the case of *Nelumbo* (Farr, 1922) and give the appearance of cell-plate. The spindle fibres are cut through as the furrowing proceeds inwards and then they disappear. Four furrows were altogether formed along with six spindles.

The formation of isobilateral tetrads (Fig. 17) seems to take place by a different method. Though strong evidence in support of the fact was not found, some of the features are suggestive of this

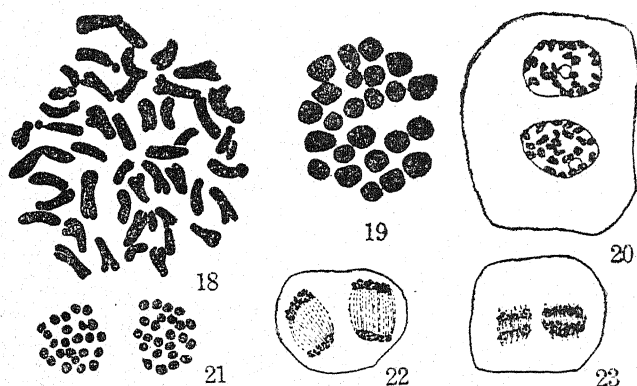
fact. Fig. 15 shows only two spindles connecting the diagonally opposite nuclei. At this stage, the spindles are situated in two dumb-bell-shaped bodies placed at right angle to each other. These two bodies appear at different foci and show apparently no connection whatever with each other, either cytoplasmic or by spindle fibres. It is not clear how such two separate bodies are formed. It may be that all the spindles except those connecting the diagonally opposite nuclei disappear and the two spindles along with the nuclei which they connect get orientated at different levels inside the cell. After this, these two spindle bodies are separated from each other presumably by a process of furrowing and give rise to two cells each containing two nuclei. The above said process is only presumed as no intermediary stages representing such furrowing were found. The result of these processes will be two cells which when they begin to undergo furrowing would give rise to the dumb-bell-shaped bodies as represented in Fig. 15.

From the literature regarding tetrad formation it cannot be gathered that any special method exists by which isobilateral tetrads are formed. But Fig. 15 suggests strongly some such special method though the intermediary stages have not been obtained.

There is no difference in the daughter-cells resulting from the two kinds of tetrad formation. The pollen grain at the shedding stage is two-celled and shows a crescent-shaped generative cell in which the cytoplasm is stained darker. The pollen when germinated in agar cultures, put out tubes without any difficulty, but no division stages were obtained.

Cytology of *N. Tabacum*

Somatic chromosomes.—48 chromosomes were counted in sections of root tips and two chromosomes with satellites were distinguished. Because of the large number it was not possible to analyse the complement (Fig. 18).



Figs. 18-23.—*Nicotiana Tabacum*. Fig. 18. Somatic metaphase plate of *Nicotiana Tabacum* showing two satellite chromosomes. $\times 3,900$. Fig. 19. Meiotic metaphase I polar view showing 24 bivalents. $\times 3,900$. Fig. 20. Interphase showing 24 chromosomes and nucleoli. $\times 2,200$. Fig. 21. M II plate. $\times 2,200$, 24/24 distribution. Figs. 22-23. Anaphase II. $\times 1,500$.

Meiosis.—Meiosis is normal as described for *glutinosa* except that 24 bivalents are formed as shown in the polar view of the M I plate (Fig. 19). The disjunction is normal.

At interkinesis 24 chromosomal bodies are seen at each pole along with a nucleolus (Fig. 20). The nucleolus disappears and the chromosomes enter into the second division stages. 24 chromosomes appear in each of the two poles in the M II plate (Fig. 21). Figs. 22 and 23 show various stages in the meiotic anaphase of the plant. The anaphase and the telophase are normal and tetrads are produced.

Viable pollen grains (Plate VI, a) are produced as a result of regular meiosis.

IV. CYTOLOGY OF THE F_1 HYBRID

Mitosis.—In sections of root tips 36 chromosomes were counted as shown in Fig. 24. Of these 12 chromosomes belong to the haploid set of *glutinosa* and the other 24 chromosomes belong to *Tabacum*. No morphological distinctions could be drawn between the chromosomes so as to enable the identification of the parental chromosomes in the somatic complement of the hybrid.

Meiosis.—From the literature regarding the meiotic behaviour of *Tabacum* \times *glutinosa* F_1 hybrid, it is known that the 36 chromosomes of the hybrid do not undergo regular pairing among themselves. But Clausen and Goodspeed (1925) mention that the chromosome behaviour of the F_1 hybrid parallels that of *N. Tabacum* \times *N. sylvestris* hybrid. In a later work Clausen (1927) reports that loose conjugation takes place between the 36 chromosomes of *glutinosa* \times *Tabacum*. This statement was in accordance with the observations of Müntzing (1935). But East (1933) in a paper about triploid *Nicotiana Tabacum* mentions, that Drosera scheme of pairing is followed in the hybrid in question. This statement was questioned by Goodspeed (1934) and was disproved by the work of Müntzing (1935). The observations recorded in this paper go to confirm the findings of Müntzing. They disclose to us the fact that a scheme of pairing representing a healthy synapsis which would result in the partial or complete fertility of the hybrid, is totally absent in this hybrid.

The hybrid far from being similar to the F_1 *Tabacum* \times *sylvestris* is thoroughly in contrast with the latter. The F_1 *Tabacum* \times *glutinosa* is completely sterile while the *sylvestris* \times *Tabacum* hybrid is partially fertile. Though Clausen and Goodspeed (1925) report partial fertility in one or two F_1 plants of *glutinosa* \times *Tabacum* hybrids, all of the plants obtained in this laboratory were invariably found to be completely sterile. The reason for this may be the difference in the variety of *Tabacum* parents used in the two cases. Greater degree of pairing is exhibited in the *sylvestris* \times *Tabacum* hybrids, while the synapsis in the case of *glutinosa* \times *Tabacum* is very weak. The case of the latter resembles in most features the behaviour of the F_1 hybrids *N. bigelovii* \times *N. glutinosa* (Goodspeed

and Clausen, 1927a). The F_1 *sylvestris* \times *tomentosa* also exhibited parallel chromosome behaviour with the *Tabacum* \times *glutinosa* hybrids (Goodspeed and Clausen, 1928).

Diakinesis.—Stages earlier than diakinesis are not described on account of the large number of chromosomes. A number of clear diakinesis stages were available from which the number and nature of the gemini could be easily determined. Müntzing (1935) determined the frequency of chromosome pairing in this hybrid by counting the number of bivalents in the first metaphase plates.

One feature regarding the successive stages of meiosis was noteworthy. Among the anthers taken from the same bud one showed first metaphase and even inter-phase, while in another, pollen mother cells were found to be in the diakinesis or pro-metaphase stages. Such a feature has been previously recorded by Goodspeed and Clausen (1927a) in interspecific hybrids having *Nicotiana bigelovii* as a parent. The authors are of opinion that "in the hybrids which show an apparently complete lack of chromosome conjugation, post-synizesis stages upto A I are, so far as we can judge, passed through with considerable rapidity". Such a gradation in the development of the pollen mother cells in the same anther-sac after zygotene stages has been observed in *Solanum melongena* (Janaki Ammal, 1934).

The degree of chromosome pairing is very limited as frequency studies of diakinesis will show in Table I. In the few cases of the bivalent formation it is due to the loose pairing between the chromosomes of *glutinosa* and those of *Tabacum*, as it has been previously proved that no pairing occurs between the chromosomes of the haploid complement in either of the two parent species. The above fact was disclosed from the studies in the haploids of *Tabacum* (Chipmann and Goodspeed, 1927) and *glutinosa* (Goodspeed and Avery, 1929).

The bivalents could be clearly distinguished by their sizes and their chiasmata. Each bivalent forms a single terminal chiasma. Such chiasmata are known to be characteristic of hybrids with minimum amount of synapsis. Goodspeed (1934) in the course of a comparative account of chiasma formation in interspecific hybrids of *Nicotiana* says "The bivalents in hybrids showing minimum amount of pairing usually form a single terminal chiasma, the total number of chiasmata equalling the total number of bivalents". So terminal chiasmata formation is a sign of weak affinity between the parental chromosomes. Cases of complete asynapsis were also met with (Fig. 30). Figs. 25-29 represent varying degrees of synapsis. Fig. 25 shows 6 bivalents and 8 was the number found to be the maximum among the 50 pollen mother cells examined. Fig. 25 shows 7 bivalents and a trivalent. Multivalent formation was most infrequent. The frequency of bivalent formation was studied from 50 different pollen mother cells. The frequency in different classes were as follows :—

TABLE I

No. of bivalents	0	1	2	3	4	5	6	7	8	Total
No. of P.M.C.s ..	2	2	6	5	19	5	5	4	2	50
Total no. of bivalents in each class ..	0	2	12	15	76	25	30	28	16	204

Average number of bivalents per *P.M.C.* 4.08.

As the average number of bivalents in one pollen mother cell is calculated to be 4 from data given in Table I, $28_I + 4_{II}$ seems to be the typical configuration at diakinesis. This seems to be corroborated by the fact of the greatest frequency occurring among 4 bivalent pollen mother cells. The observation of Mützing (1935) from first metaphase counts also confirms the above fact.

Degeneration of pollen mother cells.—Degeneration of pollen mother cells is extensive especially in the earlier stages. Many pollen mother cells are darkly stained the chromatic material having formed a dark mass. Such degenerated pollen mother cells are found even in the tetrad stages as darkly staining thick-walled and small round cells.

Cytomixis.—In some cases adjacent pollen mother cells were seen to extrude chromatic material. This feature was first observed in *Oenothera* by Gates (1911) who described such a behaviour of the meiotic nuclei just at the time of synapsis. He described how the centrally placed nucleus migrates to a peripheral position thereby causing the nuclear membrane to come in contact with the cell wall. After this the nuclear material of one cell was extruded into the cytoplasm of the adjacent cell. This nuclear extrusion named by Gates as cytomixis being finished, the nuclei of the two cells moved again to their original central position. The fate of the extruded material had been clearly explained by the author. It formed a sort of nuclear membrane inside the cell which had received it and developed into a pseudo-nucleus. Ultimately the membrane dissolved and the chromatic material was observed into the cytoplasm of the recipient cell leaving only dark patches in the places where such extruded bodies were. So according to Gates the extruded material does not go to add to the chromatic material of the recipient cell. Consequently the increase in the number of chromosomes cannot take place through cytomixis.

In many prophase stages such peripheral position of the nuclei and the aggregation of the chromatic threads towards the cell wall on the particular side were observed. Fig. 32 shows bivalents and univalents flowing along the cytoplasm into the adjacent cell. Some pollen mother cells were observed to contain along with their nuclei portions of additional nuclei (Figs. 33 and 34). Whether these additional nuclei have arisen from irregularities in pre-meiotic divisions or from cytomixis cannot be said definitely. But,

as such pollen mother cells with two nuclei were not met with in the earlier stages, it is probable that they arise through cytomixis, where only portions of nuclear matter are extruded into adjacent cells. Such binuclear pollen mother cells from irregular premeiotic divisions have been found in *Tridax* (Raghavan and Venkatasubban, 1941), where the two nuclei enter independently into the successive division stages.

Whether these additional nuclei fuse with the original one to give rise to polyploid nuclei cannot be said with certainty. Nandi (1937) describes such cases of binuclear pollen mother cell formation from cytomixis at diakinesis stages, in *Oryza*. According to him it is a method of origin of polyploidy because polyploid gametes could be produced from such binucleate pollen mother cells.

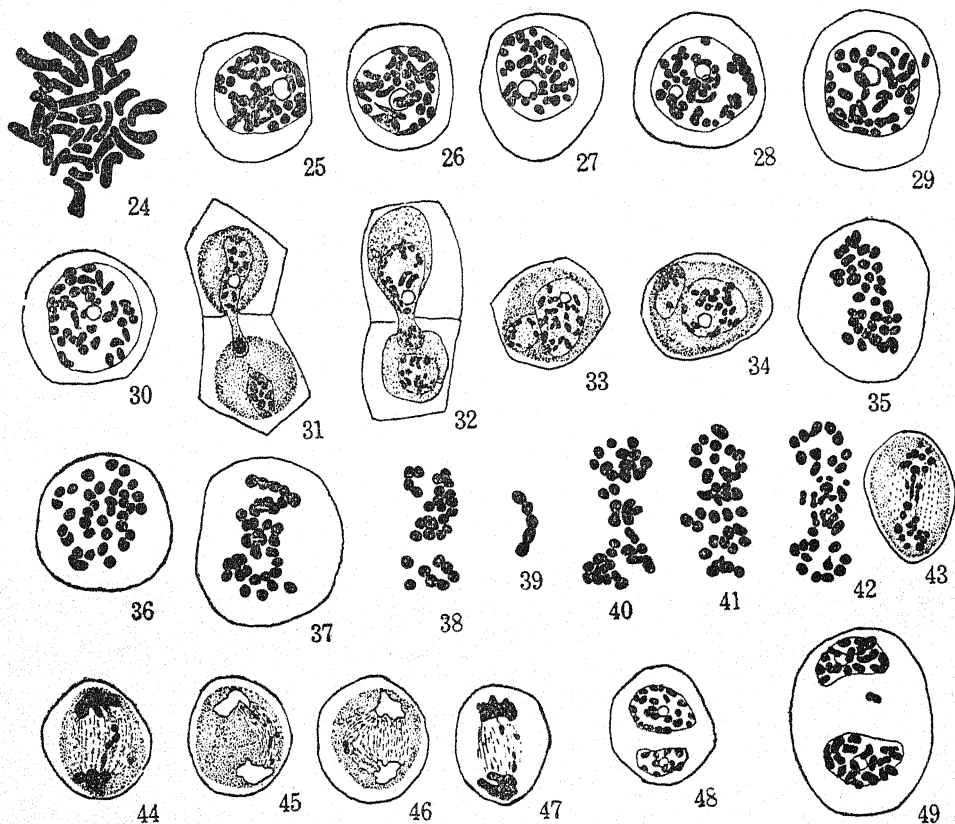
Cytomixis occurs during all stages of meiosis. Gates and Latter (1927) in *Lathraea* and Raghavan and Venkatasubban (1941) in *Tridax* have found the phenomenon at all stages of meiosis. Cytomixis has been reported in X-rayed *Capsicum* (Raghavan and Venkatasubban, 1940b). Cytomixis is probably the reason for the degeneration of pollen mother cells.

Cytomixis as an abnormality seems to be common in many hybrids. A hybrid from *Typha angustifolia* showed the phenomenon at diakinesis (Roscoe, 1927). Kattermann (1933) gives a list of the cases where this occurs and reports it in *Triticum* × *Secale* intergeneric hybrids. Percival (1930) has met with this phenomenon in *Aegilopes* × *wheat*. From this and from the evidence of Church (1929) who found the occurrence of this phenomenon very striking in connection with the hybrids of *Phalaris*, we can regard that it is more probable that this phenomenon is associated with hybrids than being merely an artefact as Sinoto (1922) will have us believe.

Fig. 31 shows apparently another case of cytomixis. In this case, instead of well-defined bivalents and univalents being extruded as in the normal case, the extruded chromatic material forms a wad. That this wad is not the nucleolus can be judged from the bigger size of the former, and from the presence of a nucleolus inside the nuclear cavity from which the extrusion takes place. A parallel case has been described by Jensen (1938) in prophase stages. He says "in some anther sacs a large wad of chromatin developed shortly after synizesis in many of the pollen mother cells. Later this lump was pushed out of the nucleus and frequently attracted a corresponding lump in the adjacent cell, which drags along with it a portion of the spireme." This according to him cannot be classified as cytomixis though it very much simulates that phenomenon. The wad formation described in the above case is similar to what has been shown in Fig. 31. But due to the absence of an attracting lump in the recipient cell the two cases cannot be said to belong to the same type of abnormality.

Prometaphase.—After diakinesis the nucleus enters into the prometaphase stage. At this time, the gemini which were distributed throughout the periphery of the nucleus during diakinesis,

come to the centre in close assemblage. Owing to this, a clumped appearance is shown by the gemini at this stage. The nuclear membrane disappears at this stage.



Figs. 24-49.—*Nicotiana glauca* × *glutinosa*. Fig. 24. Somatic metaphase plate of F_1 hybrid showing 36 chromosomes. $\times 3,900$. Figs. 25-30. Diakinesis stages. $\times 2,200$. Fig. 25. $6_{II} + 24_I$; Fig. 26. $1_{III} + 7_{II} + 19_I$; Fig. 27. $5_{II} + 26_I$; Fig. 28. $4_{II} + 28_I$; Fig. 29. $2_{II} + 32_I$; Fig. 30. 36_I . Figs. 31-32. Cytomixis. $\times 1,500$. Figs. 33-34. Binucleate pollen mother cells. $\times 1,500$. Figs. 35-41. Metaphase I. $\times 2,200$ showing the scattered condition of univalents. Figs. 35-36. $1_{II} + 34_I$; Fig. 37. $2_{II} + 32_I$ bivalents are in the centre and univalents are found scattered. Secondary associations are well seen; Fig. 38. $M I$ not representing all chromosomes shows the secondarily formed pairs and chains; Fig. 39. A secondarily formed chain of 5 univalents; Fig. 40. $2_{II} + 32_I$. The two bivalents are formed in the equatorial region; Fig. 41. 36_I . Figs. 42-47. Anaphase I. All except Fig. 2 are $\times 1,500$. Fig. 42. Showing random distribution and division of univalents. The small chromosomes are the products of such division $\times 2,200$. Fig. 43. A straight spindle. Fig. 44-45. Curved spindles showing laggards. Fig. 46. Tripolar spindle with a univalent at one of the poles. Fig. 47. Fragments at anaphase I. Figs. 48-49. Interphase. Fig. 48. Interphase with unequal nuclei. $\times 1,500$. Fig. 49. Interphase showing a univalent in the plasma. $\times 2,200$.

Metaphase I.—At first metaphase the chromosomes separate and unlike normal cases of meiosis, the chromosomes fail to arrange themselves in the equatorial plate (Fig. 35–41). They lie scattered throughout the spindle. Such a feature has been described in many cases of hybrids which show weak pairing. The bivalents occupy the equatorial portion while the univalents were in the scattered condition (Figs. 37, 40, 41). Such disposition of chromosome bodies in the MI was met with in interspecific hybrids of *Brassica* (Morinaga, 1931).

There seems to be some relationship between the degree of synapsis and the arrangement of chromosomes in the equatorial plate. In all cases where weak pairing or complete asynapsis has been reported, this scattered condition of chromosomes prevails. Many cases of haploidy could be cited to show that asynapsis and absence of a regular equatorial plate at MI go hand in hand. Haplonts of *Nicotiana Tabacum* (Chipmann and Goodspeed, 1927) and *N. glutinosa* (Goodspeed and Avery, 1929) were observed to show this feature. Catcheside (1932) accounts from such a behaviour in a haploid *Oenothera* by stating that "many of the chromosomes have never been at the equator of the spindle, but have a definite bias towards one or the other end of the poles ever since diakinesis." Humphrey (1934) reports that such cases occur in haploid tomatoes "where the chromosomes become more or less randomly distributed all over the spindle." Many examples of interspecific hybrids in the genus *Nicotiana* can be cited to show that this condition is prevalent along with weak pairing. *Nicotiana sylvestris* \times *N. tomentosa* (Goodspeed and Clausen, 1928); *N. bigelovii* \times *N. solanifolia* and *N. Tabacum* \times *N. rustica* (Goodspeed, 1934) are some of the examples.

Müntzing (1935) observed that the univalents had a tendency to stick together in pairs or chains. Such a tendency was observed in *glutinosa* \times *tomentosa* hybrids also (Elvers, 1934). In this case such pairs or chains of univalents were frequently met with during first metaphase (Figs. 37–39). Müntzing (1935) states that "it is very questionable if this phenomenon should be regarded as secondary association." But the same phenomenon observed by Catcheside (1934) in *Swede* \times *Turnip* F_1 has been referred to as secondary pairing of univalents. Moffett (1931) also found secondary association of univalents with formation of pairs. The reason for this association has been stated by him as follows: "It would seem that the chromosomes within the set are sufficiently homologous to exhibit a mutual attraction but not sufficiently homologous to pair at metaphase." Figs. 37–38 show metaphase plates where association of 2, 3 and 4 univalents are seen. In one case an association of 5 univalents was met with (Fig. 39). Richharia (1937) has found such associations of varying numbers of univalents in *Raphanus* \times *Brassica* F_1 hybrid. Such paired univalents are named as pseudogemini by Gustafsson (1935), who has recorded this phenomenon in the *E.M.C.* of *Taraxacum* and gives the following explanation for their origin. "The formation of pseudogemini is due to the fact that single homologous chromosomes happen to lie in the

vicinity of each other during diakinesis and that they arise when the repulsion between the chromosomes on the passage to the metaphase decreases and attraction becomes too powerful."

Considering the above mentioned cases which closely resemble what has been found in the present hybrid, it seems most likely that the chains and pairs of chromosomes found at first metaphase are due to secondary association between univalents. As to their origin, it may be said that the chromosomes which fail to form chiasmata and give rise to normal bivalents, because of their weak homology probably form secondary associations at first metaphase.

Anaphase I.—Shows the disjunction of bivalents and random distribution of univalents. Some of the univalents undergo division at this stage. Fig. 42 shows 6 small bodies along with univalents which have not reached the poles. These are probably the products of division of three univalents. Division of univalents is a feature described in some other hybrids also which show such weak pairing.

Tackholm (1922) classified the irregularities of meiosis in hybrids with unequal parental chromosome numbers into three groups. (1) The *Drosera* scheme with strong affinity, (2) The *Hieracium* \times *Boreale* scheme with weak affinity, (3) The *Pygerarea* scheme with no affinity. Each of these schemes was subdivided into three classes according to the behaviour of univalents in the respective cases. In the first case, all the univalents are distributed intact and at random to the poles, secondly random distribution takes place along with division of some of the univalents and lastly all univalents undergo division, and split halves are distributed to the poles. Of these cases the present hybrid belongs to the second type in *Hieracium Boreale* scheme, because of the weak affinity and division of some of the univalents.

Frequently, univalents and fragments are seen to lag in a spindle (Fig. 44). In Fig. 45 the spindle is much curved. Fig. 46 shows probably a tripolar spindle. The curved spindle is the reason for some of the univalents being left off in the plasm. The distribution first takes place along the straight spindles and when in late anaphase the curved spindles are formed, frequently the gemini which are drawn along these later formed spindle, fail to reach the poles and thus get stranded in the plasma. Such tripolar spindles and curved spindles have been met with in the case of *Nicotiana bigelovii* \times *glutinosa* hybrids.

As the univalents are distributed at random, in many cases, one of the poles gets a greater number of univalents than the other as will be seen in M II studies later.

Interphase.—Like the parents the hybrid has also got a well-defined interphase when the two chromatin masses resulting from the first division organise into two nuclei at the two poles. In the interphase frequently the two daughter nuclei are sometimes unequal in size and contain unequal number of chromosomes (Fig. 48). This is presumably due to the random distribution followed in the first

anaphase. The laggards of A I are found in the plasma during the interphase and are never included in either of the two nuclei. The laggards were univalents and fragments. These did not organise into micronuclei except in cases where wall formation followed after first division as an abnormality (Fig. 50). The fate of these laggards could not be ascertained. Either they persist throughout the second division and get included in any one of the four second telophase nuclei or they get lost in the cytoplasm. A fairly high percentage of cells are seen to contain such bodies in the plasma. Out of 100 cells examined, about 53 cells show such bodies. Fig. 49 shows a univalent in the plasma between the two interphase nuclei.

The interphase is an important stage as it denotes one of the probable methods of origin of dyads in this hybrid. Just before the interphase nuclei are being organised and when the spindle persists, a cleavage is effected in the centre of the cell and two daughter cells result, each of the cells containing a nucleus. These two cells probably do not take part in the second division. Wall formation after the first division is not a common feature in dicotyledons. It is an abnormality. This fact and the nature of the nuclei make us believe, that there will be no second division. Thus dyads may arise. Figs. 50-51 show how the wall formation proceeds by a process of furrowing. In Fig. 50 one cell contains a micronucleus probably organised from the lagging univalents.

Laggards did not form a nuclear membrane in ordinary cases of interphase. Such micronuclei are formed only in cases where wall formation followed the first division. The reason for this is not quite clear but it is likely that the formation of micronuclei in such cases denotes an end of the division activity of the pollen mother cell at this stage. The two daughter cells were not seen to round off as two pollen grains and it cannot be said definitely that these dyads form part of the dyads which occur along with tetrads and which arise by other methods.

Second metaphase.—The second metaphase plates (Figs. 54-61) show frequently more than the expected 36 chromosomes. Many plates at M II were counted to study the frequency of chromosomes in a single M II plate. The results are tabulated below :—

TABLE II

No. of chromosomes in a single M II plate	13	14	15	16	17	18	19	20	21	22	23	24	25
No. of cells ..	2	1	5	5	11	6	7	7	12	2	1	0	1
Total No. of chromosomes in each class	26	14	75	80	187	108	133	140	252	44	23	0	25

Total No. of P.M.C.s 60.

Total number of chromosomes 1107.

Average number of chromosomes per single plate 18.45.

In one case, one of the M II plates was seen to contain 25 chromosomes (Fig. 57). Many cells show 21 chromosomes in a single plate. Frequently univalents or laggards were seen lying in the plasma. Fig. 58 shows a fragment in the plasma and Fig. 59 shows a full univalent. These bodies are those which are left at AI and which have persisted upto the second division without having been lost. Whether these bodies in any way hinder or interrupt the second division could not be definitely stated. No cases were seen where they connected the two metaphase plates by forming bridges between them. Usually these occupy a peripheral position in the equatorial region of the pollen mother cell.

Total number of chromosomes in the two M II plates frequently vary much from the expected number. In many cases more than 36 chromosomes were found in both the plates. The average number of chromosomes calculated from Table II is higher than 18, which should be the normal average number of chromosomes in one pole of the M II plate. According to this the average total number of chromosomes during M II should be 37 instead of 36. This increased number is probably due to the division of univalents during the first anaphase. Fig. 57 shows chromosomes in the P.M.C. In these cases the small chromosomes should be taken as the daughter chromosomes of the univalents.

Fig. 55 shows a regular M II stage where each of the two M II plates contains 18 chromosomes. Such cases were however not very frequent as indicated by Table II. The occurrence of widely varying numbers of chromosomes in the two plates has been reported in the case of the hybrids *Nicotiana bigelovii* \times *N. suaveolens* and *N. bigelovii* \times *N. glutinosa* (Goodspeed and Clausen, 1927a). Such a distribution of chromosomes may be the reason for the formation of polymorphic pollen.

Fig. 60 and more markedly Fig. 61, suggest the fusion of two metaphase plates. Fig. 62 is considered to be the result of such a fusion. Fusion of second metaphase plates and subsequent dyad formation have been reported in many cases. In X-rayed *Nicotiana* Goodspeed (1929) has reported such a fusion. Morinaga (1931) has observed this phenomenon and dyad formation in interspecific hybrids of *Brassica*. Recently Raghavan and Venkatasubban (1940b) have recorded the fusion of M II plates in X-rayed *Capsicum*.

Fig. 63 shows a giant spindle which is not to be held as belonging to the first division, for, more than 36 chromosomes could be counted in the anaphase. This spindle might have formed in two ways. It might have originated from the fusion of two metaphase plates into a giant metaphase plate and its subsequent division or through the fusion of two A II spindles arranged parallel to each other side by side. In whatever way they might have arisen there is no doubt that these will produce only two daughter nuclei except for some microcytes, organised by the lagging fragments and univalents. Fig. 64 is another case where the daughter chromosomes

are proceeding up the giant spindles. This is one of the ways by which dyad formation may take place.

Sometimes chromosome groups are connected together by long chromosomes forming bridges. The exact nature of these chromosomes forming these bridges and the reasons for the bridge formation could not be studied in detail. The bridge formation is probably the cause of structural alteration of the chromosomes during the divisions and if this is true this case goes to support the theory that changes in chromosome structure often take place during meiosis in species hybrids. Müntzing (1935) in *Nicotiana bonariensis* \times *N. Longsdorffi* hybrids give support to the above said theory.

From the above mentioned facts it is clear that dyad formation may take place by various methods. Formation of polyploid gametes like dyads and monads will be discussed in detail later. That dyads are formed in this case is proved beyond any doubt from the behaviour of chromosomes during the second division and from the wall formation following the first division. Müntzing (1935) stated that no dyads whatever were seen in the hybrid specimens which he investigated but in this case there is overwhelming evidence to prove that dyads are formed. Whether these dyads form viable pollen grains is doubtful. This difference between these two cases is probably due to the difference in the variety of the *Tabacum* parents used. Acetocarmine preparations show dyads along with tetrads though the percentage of the former is very low. Figs. 52-53 are from acetocarmine preparations. In one slide many sections show at least a single dyad in each anther-sac. Moreover, dyad formation is not an uncommon occurrence in interspecific hybrids. Like the arrangement of chromosomes in the first metaphase, dyad formation also is found associated with cases of asynapsis. In haploids or species hybrids where asynapsis or weak pairing occurs dyad formation also is met with. Goodspeed and Clausen (1928) report the presence of dyads in the hybrids of *tomentosa* \times *sylvestris* which exhibit parallel cytological behaviour with *Tabacum* \times *glutinosa* hybrid. There, he states "dyad production is not an uncommon occurrence in *Nicotiana* hybrids and while it appears characteristic of the hybrids in which the chromosomes fail to conjugate, it is also observed in those which exhibit the Drosera scheme of conjugation." This statement and the facts quoted above will strengthen the view that, as this is a case of weak synapsis, dyad formation also is naturally associated with it, though it was absent in the specimens of the hybrids which Müntzing examined.

Apart from dyads, microcytes are also formed. Their origin is also due to the other irregularities in the second anaphase. In normal cases 18 chromosomes should be found in each of the 4 telophase groups. In Fig. 67, two of the groups contain 18 and of the other two, one has got 17 and the other 19. But even this case should be considered to be the nearest to the normal distribution. In Fig. 68, 4 definite telophase groups are absent and in the same group sets of chromosomes have arranged themselves too distant



Figs. 50-82.—*F₁ Hybrid*. Figs. 50-51. Showing wall formation after the first division. Fig. 50. Shows a micronucleus in one of the cells. $\times 1,500$. Figs. 52-53. Dyads occurring inter mixed with tetrad from acetocarmine preparations. $\times 1,200$. Figs. 54-61. Second metaphase. $\times 2,200$. Fig. 54. 20/17; Fig. 55. 18/18; Fig. 56. 16/23; Fig. 57. 13/25; Fig. 58. 21/15; showing a fragment in the plasma between the plates. Fig. 59. 10/27 and an univalent in the plasma; Fig. 60. 18/17. Fig. 61. The two metaphase plates have more or less fused. Fig. 62. Probably a giant metaphase caused by fusion of two metaphase plates. $\times 420$. Figs. 63-64. Giant spindles. Fig. 63. $\times 2,200$; Fig. 64. $\times 420$. Fig. 65. Second anaphase and fragments. $\times 2,200$. Fig. 66. Second anaphase bridge connecting bridges two groups of chromosomes. $\times 420$. Figs. 67-68. Second telophase. Fig. 67. Shows four groups. $\times 2,200$. Fig. 68. Not showing 4 distinct groups. $\times 1,500$. Figs. 69-76. Bridge formation and fragmentation. $\times 700$. Figs. 77-78. Probably show fusion of telophase nuclei. $\times 700$. Figs. 79-80. Tetrads with two small cells. $\times 350$. Fig. 81. P.M.C. with seven cells. $\times 700$. Fig. 82. A pentad. $\times 350$.

from each other to permit the inclusion of all of them in one and the same nucleus. So each set comes to have a nuclear membrane and a cell wall giving rise to pentads or tetrads containing more cells. A pentad and a seven-celled P.M.C. have been figured in Figs. 82 and 81 respectively.

Fragments are produced from lagging chromosomes which sometimes form bridges. When the leggards ultimately join any of the telophase groups they get split and give rise to fragments. These fragments may form microcytes. Figs. 69-72 show chromosome lagging and fragmentation. Figs. 73-76 show telophase groups of unequal size and fragments from which micronuclei and microcytes may arise. Figs. 79-80 show tetrads in which two of the cells are very small in size having probably been formed from the fragments.

Micronuclei formation of this sort has been described in *Nicotiana bigelovii* × *N. suaveolens* hybrids (Goodspeed and Clausen, 1927a). This phenomenon has been reported in many triploids. Raghavan and Venkatasubban (1940a) have recorded it in triploid *Urginea*. Dark (1932) has observed it in triploid *Hemerocallis* and is of opinion that these arise from univalents which are situated too distant to be included with the other chromosome. Takenaka (1929) is of opinion that these arise from stray chromosomes around which a nuclear membrane and a cell wall are formed. Frequency studies of tetrads with microcytes and dyads was done and the result was as follows :—

TABLE III

	Normal tetrads	Tetrads with one microcyte	Tetrads with more than one microcyte	Dyads	Total No. of tetrads
No. of cases ..	102	110	31	7	250
Percentage ..	40.8	44	12.4	2.8	100

More than 56% of the tetrads have microcytes, the remaining cells being called normal. They are normal tetrads only inasmuch as they have got 4 cells, neither more nor less. Very frequently the 4 cells show great disparity in their sizes (Figs. 79-80).

Pollen grains, as may be expected from the above mentioned facts, are polymorphic (Pl. VI, b). There is no strict demarcation of sizes. All sizes are found and most of the pollen seem to be non-viable. When treated with acetocarmine, none except a very few grains took up the stain. Figs. a and b in Plate VI show the microphotographs of the pollen of the *Tabacum* parent and the hybrid respectively. Germination of the pollen in agar cultures as was done for *Nemophila* (Wulff and Raghavan, 1937) was tried in this case. Not even a single pollen tube was put out. But this time

no control experiments with the pollen of the parents were done. The unhealthy appearance of the pollen and their failure to germinate in the pollen tube cultures clearly indicate that these pollen are not viable.

At the shedding time the generative cell is formed in the good pollen which was seen in the centre like a crescent moon. But the number of such good pollen is very low when compared with the non-viable pollen grains. Moreover, there is not much evidence to show that even these good pollen grains are viable. Crossing experiments using the pollen of the hybrid on the stigmas of the parents are being done. Until the results prove to us definitely it cannot be said with certainty that the microspore of *glutinosa* \times *Tabacum* F_1 is viable.

V. DISCUSSION

(a) Interspecific hybridisation — a guide to ancestral homology

Interspecific hybridisation and the study of interspecific and intergeneric hybrids have long been engaging the attention of many cytologists, because the results obtained by these studies serve as valuable clues to determine the relationship between the various species. The mode of origin of new species can be established with the help of interspecific hybridisation. Species having the same number of chromosomes when crossed with each other will give either fertile hybrids or hybrids of a partially or completely sterile nature. Such fertile hybrids are met with in the cases of *Viola* (Clausen, J., 1931), *Nicotiana* (Goodspeed, 1934) and *Triticum* (Aase, 1930). Cytological investigation of these hybrids revealed regular pairing between the parental chromosomes. This complete synapsis is the reason for fertility of these hybrids and indicates that the homology of the two sets of chromosomes is complete. Therefore, the two parents which are taxonomically distinguished as two different species can be traced back to the same origin cytologically.

In the case of sterile hybrids, some show partial pairing with formation of varying numbers of bivalents and univalents. The greater the number of bivalents the more fertile is the hybrid. Such cases occur in *Nicotiana* (Goodspeed, 1934), *Viola* (Clausen, J., 1931) and *Brassica* (Morinaga, 1929a and 1931). There are other cases where no pairing whatever has been observed. This complete asynapsis is characteristic of intergeneric hybrids, e.g., *Raphanus sativus* \times *Brassica oleracea* (Karpechenko, 1927, 1928) and *Aegilops ovata* \times *Triticum diococcum* (Sax, 1928). Examples of interspecific hybrids with complete asynapsis are not rare, e.g., *Crepis* (Collins and Mann, 1923) *Digitalis* (Hassebessel, 1916) *Nicotiana* (Goodspeed, 1934).

So far hybrids whose parents have the same number of chromosomes have been considered. The cases of hybrids whose parents possess different number of chromosomes are more complicated and none the less interesting. In some cases varying number of bivalents are formed the number of such bivalents generally being

equal to the haploid chromosome number of the parent with the smaller number of chromosomes. This belongs to the Drosera scheme of pairing according to Tackholm (1922). *Nicotiana Tabacum* \times *N. sylvestris* hybrids (Goodspeed and Clausen, 1927b) exhibited $12_{II} + 12_I$. Sax found in 1928 that from a cross of *Triticum durum* ($n=14$) \times *Triticum vulgare* ($n=21$) in the F_1 a $14_{II} + 7_I$ configuration was obtained. Clausen, J. (1931) observed $13_{II} + 4_I$ in the hybrid *Viola arvensis* ($n=17$) \times *Viola tricolor* ($n=13$). Goodspeed and Clausen (1927b) make mention of a cross between *Nicotiana longiflora* ($n=10$) \times *Nicotiana alata* ($n=9$) giving rise to a hybrid showing $9_{II} + 1_I$. All these data regarding the number of bivalents in the hybrid, denote the number of homologous chromosomes in the parents and as such, those chromosomes which thus undergo synapsis, should have more or less the same composition and could be traced back to identical origin. So these data are valuable in drawing up schemes of composition and origin of chromosome sets in species.

Another usefulness of interspecific hybridisation is the synthesis of new species. Many sterile hybrids, both interspecific and intergeneric, are known to have become fertile by doubling their chromosomes. Thus, amphidiploids arise. *Raphanobrassica* (Karpechenko, 1927) is an example of an amphidiploid from an intergeneric hybrid. Many examples of amphidiploids from interspecific hybrids could be cited. Winge in 1932 has reported a number of such cases. Nilsson (1925) got a sterile hybrid having 42 chromosomes between *Festuca arundinacea* \times *F. gigantea*. This hybrid when backcrossed to one of the parents gave rise to an amphidiploid with 84 chromosomes. Müntzing (1930) synthesized the naturally occurring *Galeopsis tetrahit* from the two species, *Galeopsis pubescens* and *Galeopsis speciosa*. The work of Huskins (1931) established on cytological grounds that *Spartina Townsendii* is an amphidiploid. Lastly there is the evidence of Kostoff (1936) as to the origin of *N. rustica* as an amphidiploid from *Nicotiana paniculata* \times *N. undulata*. Goodspeed and Clausen (1928) have similarly proved the origin of *N. Tabacum* as an amphidiploid from *N. sylvestris* \times *N. tomentosa*. All these go to substantiate that, amphidiploid derivatives behave like new stable species and that their origin is from intergeneric and interspecific hybrids.

In some other cases all the chromosomes of both the parents are not included in such hybrid derivatives. Only certain chromosomes of one parent are combined with the chromosomes of the other parent. Such cases are to be found in *Viola hyperchromatica* having 42 to 45 chromosomes from a cross by Clausen, J. (1926) between *Viola tricolor* ($n=13$) \times *V. arvensis* ($n=17$). Webber in 1930 got a 50 chromosome derivative from *N. sylvestris* \times *N. Tabacum* hybrid by selfing it. Lammerts (1932) obtained in a similar manner a 60 chromosome derivative from *N. rustica* \times *N. paniculata* hybrid. All these derivatives bred true and behaved like constant species showing no signs of their hybridity.

The above mentioned cases are all new derivatives with changes in chromosome numbers only. Derivatives with changes in chromosome morphology have also been met with as an example of which can be mentioned *Crepis*. The works of Navaschin (1927, 1933 and 1934) give us a lot of evidence on this matter. The view that such structural changes are effected during the meiosis of the hybrid was expressed by Müntzing also (1935).

So these facts show clearly that interspecific hybrids reveal the relationship between the varicous species and also afford ample opportunities for the evolution of new species through chromosomal changes both numerical and structural.

The present cross effected between *Tabacum* ($2n = 48$) and *glutinosa* ($2n = 24$) would appear to exhibit practically no pairing. The 36 bodies appear as univalents in diakinesis and first metaphase of the meiosis of the pollen mother cells. Bivalents are formed only to a limited extent. On an average 4 bivalents are formed in each pollen mother cell according to the data given in Table I. The factors that are responsible for this partial synapsis are not quite clear.

Goodspeed (1934) has utilised cytological evidence to elucidate interpretation of the relation of chromosome behaviour in interspecific hybrids to phylaxis from observation over the behaviour of a number of interspecific hybrids in *Nicotiana*. The following was one of the conclusions that was arrived at. "The minimum amount of pairing bears a definite relationship to the number of chromosomes and may be a reflection of (a) the residual homology possessed by the races, the progenitors of which were more closely related to them than to-day; (b) the occurrence of non-homologous association at pachytene."

In two of the pollen mother cells 8 bivalents were seen in the present hybrid. The pairing in this case cannot be called conjugation of the Drosera scheme as it would involve the formation of at least 12 bivalents. It may be said that it is a partial autosyndesis where 16 chromosomes of *Tabacum* have chosen to synapse and form 8 bivalents. But against this we have to remember the fact that haploid *Tabacum* which appeared in the cultures of Chipmann and Goodspeed (1927) showed no pairing at all.

Since *tabacum* has an amphidiploid of *tomentosa* \times *sylvestris* as its progenitor, the 24 haploid chromosomes of *Tabacum* may be regarded to be composed of 12 *tomentosa* and 12 *sylvestris* chromosomes. That these will not pair among themselves is clear from the fact that *tabacum* is an amphidiploid. No synapsis taking place in the *tomentosa* \times *sylvestris* hybrid, it is likely that the bivalents formed are those of *sylvestris* or *tomentosa* chromosomes at random with *glutinosa*.

The behaviour of *tomentosa* \times *glutinosa* hybrid throws much light on the nature of pairing in the present case. The *tomentosa* \times *glutinosa* hybrid has 24 chromosomes of which the frequent formation of 5 bivalents has been reported (Elvers, 1934; Goodspeed,

1934). As *tomentosa* forms part of the haploid chromosome complement of *Tabacum* the pairing seen in the present hybrid is probably between *glutinosa* chromosomes and *tomentosa* portion of *Tabacum* chromosomes. Even the abnormal case of 8 bivalents can be explained on this basis. For according to both the authors quoted above, as many as 9 bivalents are formed in some cases in the *tomentosa* \times *glutinosa* hybrid. The nature of pairing in the latter hybrid not being quite clear it is impossible to state positively that the synapsis is between the *glutinosa* chromosomes and *tomentosa* part of the *Tabacum* chromosomes.

One fact stands prominently from this cross and that is the comparative distance between *Tabacum* and *glutinosa* species. This is mainly inferred from this almost complete lack of synapsis referred to above. Amphidiploids from this hybrid have been obtained as bud sports (Clausen and Goodspeed, 1925). In this allopolyploid *Nicotiana digluta*, Clausen and Goodspeed (1925) found only autosyndesis. Degeneration in this hybrid is extensive. Embryo-sac is not formed beyond the uninucleate stage and the pollen is completely sterile and non-viable (Baghavan and Srinivasan, A. R., 1941).

So there can be no question of amphidiploids arising sexually. It can only be by somatic doubling. It has already been said that *Tabacum* is an amphidiploid of *sylvestris* \times *tomentosa*. They are not closely related inasmuch as there is no pairing of chromosomes in the hybrid between them. Similarly between *Tabacum* and *glutinosa* there is practically no pairing indicating a distant homology, though considering the degree of pairing in both the cases *glutinosa* may be said to be more related to *Tabacum* than *sylvestris* and *tomentosa* are to each other.

Evidence has been cited above to show the probability of synapsis being between *glutinosa* and *tomentosa* portion of *Tabacum* chromosomes. Whether *sylvestris* and *glutinosa* chromosomes are closely related and could synapse can be inferred from the data about chromosome pairing in the *glutinosa* \times *sylvestris* hybrids (Goodspeed, 1934).

In the above case the amount of pairing is considerably less than what we get between *tomentosa* and *glutinosa*. A maximum of 4 bivalents are formed in *sylvestris* as against 9 in *tomentosa* and most of the pollen mother cells show only two bivalents in the former as against 5 of the latter. So it follows that *glutinosa* is more related to *tomentosa* than to *sylvestris* and hence if any allosyndetic pairing occurs, it is more likely to be between *glutinosa* and *tomentosa* than between *glutinosa* and *sylvestris*.

The abovesaid fact agrees well with the classification of the *Nicotiana* species by Goodspeed (1933). *Glutinosa* has been included in the *tomentosa* group on the basis of similarity in chromosome number and morphology, while *sylvestris* has been separated from this group though it has got the same number of chromosomes as *tomentosa*. It is possible that larger differences in chromosome

morphology in the case of *sylvestris* make pairing more difficult than in the case of *glutinosa*.

(b) *Formation of Polyploid gametes*

Formation of dyads has been observed in the present hybrid and the probable methods by which they are formed have also been described. The production of dyads in unreduced gametes leads to the establishment of polyploidy sexually. Polyploid gamete formation has been reported in many cases. Many interspecific hybrids as an example of which may be cited *Brassica* (Morinaga, 1931), produce dyads the origin of which has been ascribed to the fusion of second metaphase plates.

Polyploid gamete formation has been studied in *Avena* by Ellison (1937). But *Avena* is a monocot and details cannot be common to that plant and *Nicotiana* which belongs to the dicotyledons. But the essential feature that was observed by her was the failure of cell-wall developments at various stages where wall formation ought to have taken place during normal meiosis.

This absence of wall formation in premeiotic divisions will result in pollen mother cells with two nuclei. If these two nuclei fuse we will get a nucleus which has a tetraploid number of chromosomes and will give rise to diploid gametes assuming that meiosis takes place normally. Binucleate pollen mother cells thus produced have been reported in *Tridax* (Raghavan and Venkatasubban, 1941). Such binucleate pollen mother cells are also formed as a result of cytomixis as in the present case in *Oryza* (Nandi, 1937).

Binucleate pollen mother cells were met with in this case also and it is more probable that they arose through cytomixis than through suppression of wall formation during the premeiotic divisions. As the binucleate pollen mother cells have an increased number of chromosomes they are sure to produce polyploid gametes if the meiosis is regular.

If wall formation takes place after the first division and the second division is suppressed as is presumed in some of the cases of the present hybrid, polyploid gametes cannot arise. If however, the nuclei of the two daughter cells divide without subsequent cell wall formation then dyads with diploid number of chromosomes will be produced assuming that the chromosomes at A I have been equally distributed to the two poles. The suppression of the second division and the phenomenon of "monokinetic division" has been observed by Levan (1933) in *Allium* species but in that case no wall formation was observed and the result was a giant pollen grain. So it is evident that the dyad-like bodies seen during interphase in this case do not produce polyploid gametes.

The fusion of M II plates has been uniformly found in almost all cases where polyploid gametes arise. In the present hybrid also such a phenomenon was observed. In monocotyledons suppression of wall formation after the first division is necessary to ensure the

fusion of M II plates. But in dicotyledons there is no cell-wall formation after the first division and the fusion is much more easily possible. Dyads resulting from such fused giant M II plates will contain polyploid numbers of chromosomes. If wall formation is suppressed after this division a monad which contains $4n$ chromosomes will result. Monads in the case of *Seilla* (Raghavan and Venkatasubban, 1939) were obtained by fusion of 4 haploid telophase groups of chromosomes owing to the suppression of wall formation in the preceding divisions. Such fusions are also found in this case and denote probably another method by which polyploid gametes arise (Figs. 77-78).

So it is evident that suppression of wall formation is the chief feature in the production of polyploid gametes. Formation of restitution nucleus is also one of the methods of production of diploid gametes and has been reported in many cases.

In whatever way these gametes may arise it has been observed that only very few of these gametes are fertile. In this case almost all the pollen were observed to be non-viable. But some viable polyploid pollen also are present though to a very low percentage. In order to test the viability of such pollen, back-crossing experiments with the parents are carried on and only from the results we can ascertain the polyploid nature of the few viable pollen that are produced by this hybrid.

VI. SUMMARY

1. Interspecific crosses between a South Indian variety of *Nicotiana Tabacum* and *N. glutinosa*, were effected and the cytology of the hybrid was studied for the first time in India.

2. Details of meiosis in the *glutinosa* parent have been worked out and the process of tetrad formation by furrowing is described. The meiotic features of the particular variety of *Tabacum* have also been recorded.

3. The phenomenon of cytomixis has been described and the light that it throws on polyploidy is discussed.

4. The scattered arrangement of chromosomes at the first metaphase I has been discussed in relation to haploidy and asynaptic interspecific hybrids; "secondary pairing" of univalents has been recorded.

5. Dyad formation is reported and the various methods by which it is formed are described.

6. Pollen grains are generally non-viable but a few good pollen are produced which are probably polyploid and these are two celled at shedding time.

7. Interspecific hybridization is discussed as a guide to ancestral homology. The synaptic behaviour of the present hybrid is discussed in this light.

8. Polyploid gamete formation has been discussed in the light of observations made about dyad formation in the present case.

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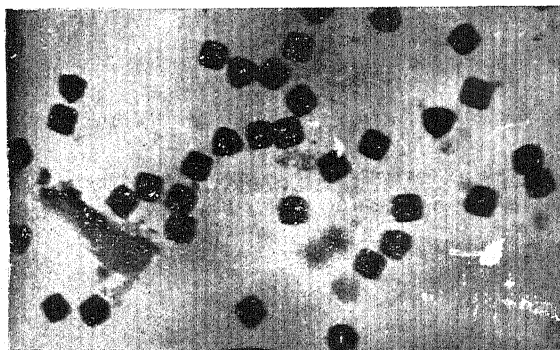
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EXPLANATION OF PLATE VI

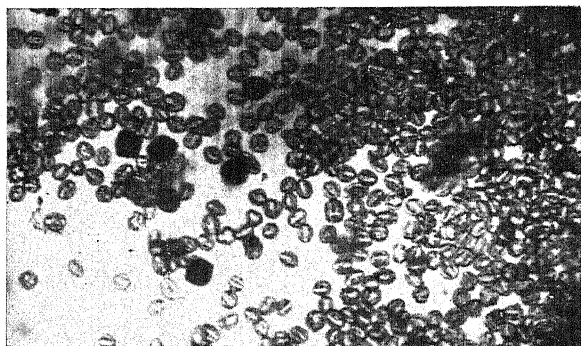
(a) Microphotograph of the pollen of the *Tabacum* parent.

(b) Microphotograph of the pollen of *N. Tabacum* × *N. glutinosa* F₁ hybrid. Many of the pollen have not been stained. Only very few viable pollen have taken up the stains.

Both are from acetocarmine preparations.



a



b

T. S. RAGHAVAN
AND
A. R. SRINIVASAN

CYTOGENETICAL STUDIES IN NICOTIANA

STUDIES IN THE RUBIACEÆ

Part I. Development of female gametophyte and embryo formation in *Dentella repens* Forst. and *Oldenlandia alata* Koch. and some cyto-taxonomical considerations

By T. S. RAGHAVAN, M.A., PH.D. (LOND.), F.L.S.

AND

K. RANGASWAMY, M.Sc.

Annamalai University

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1. INTRODUCTION

EARLIEST workers in this family are Schleiden (1837) and Hofmeister (1858). The works of these authors have been summarised and many of their views were modified by Lloyd (1902). Schnarf (1931) gives a list of other authors, who have investigated chiefly the members of the temperate genera belonging to this family.

The first work of much significance was by Lloyd (1902) who compared the embryological features of many genera in this family. His work deals with information about the genera *Vaillantia*, *Callipeltis*, *Asperula*, *Crucianella*, *Diodia*, *Sherardia*, *Rubia*, *Richardsonia* and *Houstonia*. He has described the ovule of *Houstonia* as having no integuments. His work throws much light on the nature of nucellus in the plants belonging to this natural order. The occurrence of suspensor and antipodal haustoria, has also been reported in many genera. The cytology and the behaviour of pollen tube in certain genera have been described.

The work of Eagerlind (1937) is one of much importance after Lloyd's. Eagerlind has studied, in addition to those genera investigated by Lloyd, some more temperate genera and some tropical genera like *Pavetta*, *Ixora*, etc. He has described the embryology of *Spermacoe tenoir* and an unspecified member of the genus *Oldenlandia*. He has divided the family into many groups which exhibit typical methods of integument and nucellus development, embryo-sac formation, antipodal haustoria development, etc. Chromosome numbers of a large number of species distributed over various genera are given, with special reference to the genus *Galia* and the light it throws on polyploidy.

Stevens (1912) has described the cytology of *Houstonia* and Houk (1938) has investigated the development of integument and nucellus in *Coffea*. The latter author has mentioned that a structure called the obturator envelops the young ovule and disintegrates as the ovule grows in size. Krug (1937) has studied the genus *Coffea* cytogenetically.

In the present work the embryological features of *Dentella repens* and *Oldenlandia alata* are described. The chromosome numbers of some species have been reported and an attempt is made to discuss the basic number of chromosomes in Rubiaceæ.

Dentella and *Oldenlandia* are herbs belonging to the Hedyoteae tribe of the Rubiaceæ. These are generally found in moist places on the edge of canals and pools.

2. MATERIALS AND METHODS

Somatic chromosome counts were made from root-tips fixed in Navaschin's fluid. Maximum mitotic activity was observed between 9 and 10 A.M. For meiotic studies, the correct stage for fixation of anthers was determined by acetocarmine examination. Where the anthers were very small, whole buds were prefixed in Carnoy's fluid for 2-5 seconds and then transferred to Navaschin's chrom-acetic-formalin.

For morphological studies the ovaries were fixed in Formalin Acetic Alcohol. The materials were dehydrated as usual and then imbedded in paraffin wax using chloroform as the paraffin solvent. Sections were taken at thicknesses varying from 6-15 microns and stained in Newton's Iodine Gentian-Violet and Haidenhein's Iron-Alum Hematoxylin.

3. DENTELLA REPENS Forst.

The ovary is inferior and bi-locular with an axile placenta bearing numerous ovules (Fig. 1). The ovules at first arise as straight protuberances from the placenta and then they curve towards one side. The archesporium is differentiated at this period and it consists of either a single cell or two cells differentiated from the hypodermal layer. The nuclei of these are bigger in size and the cell is rich in cytoplasm. Sometimes, two archesporial cells

arise one below the other (Fig. 2). As the ovule grows in size the archesporium gradually elongates and the body of the ovule becomes bent and at a later stage, when the archesporium has attained its maximum size, the body of the ovule lies at right angles or a little wider angle to the funicle. Neither the body of the ovule nor the embryo-sac is curved (Fig. 1). The ovule can neither be classed as campylotropous nor anatropous. It is midway between orthotropous and amphitropous types, as the ovule at a later stage makes a wider angle with the funicle.

The megaspore mother cell has a single epidermal cell covering it at its apex, and on all other sides, is surrounded by the massive single integument (Fig. 4). No nucellar layer is found to cover the archesporium on all sides as is met with in Solanaceæ, Scrophulariaceæ, etc. The single epidermal cell probably represents the much reduced nucellus. The nature of nucellus and integument in the plants of Rubiaceæ is not quite clear and will be discussed in detail later. Such epidermal nucelli are otherwise known as the 'rudimentary nucelli', and are met with in Apocyanaceæ also (Anderson, 1931).

With the development of the ovule, the cells of the integument divide vigorously and as a result the integuments grow past the single epidermal nucellar cell, which does not divide. The cells of the integument are closely set and the micropylar canal is very delicate. In later stages than the dyad stage, the micropylar canal could not be made out. This was perhaps the reason why the ovules of Rubiaceæ were thought to be consisting of a naked nucellus (nucellus nudus), i.e., without any integument, by Schleiden (1837).

The single megaspore mother cell undergoes reduction division and gives rise to the dyads (Figs. 5 and 6), from which a linear tetrad is formed (Fig. 7). Though two archesporial initials were observed in some cases, only one developed into the megaspore mother cell. The development of more than one archesporium up to the tetrad stage is not rare in Rubiaceæ. Lloyd (1902) has reported such cases in *Callipeltis*, *Galium* and *Crucianella*.

Of the four cells of the tetrad only one cell developed. In *Crucianella*, all the four cells of the tetrads were found to develop into embryo-sacs (Lloyd, 1902). Such a condition has been met with in *Alchemilla* also, where two or three of the megaspores arising from a single archesporium, develop simultaneously (Mürbeck, 1901). In *Dentella* however, the chalazal cell developed and the other three degenerated (Fig. 8). At this stage, the cells of the integument immediately surrounding the megaspore frequently degenerate owing to the growth of the megaspore into the uninucleate embryo-sac. Lengthening of the megaspore and vacuolation results in the uninucleate embryo-sac (Fig. 9). The single nucleus now divides thrice resulting in the eight nucleate embryo-sac. The formation of the mature embryo-sac from three divisions of the megaspore is characteristic of the normal type of embryo-sac formation. Though normal type is the rule, *Scilla* type of

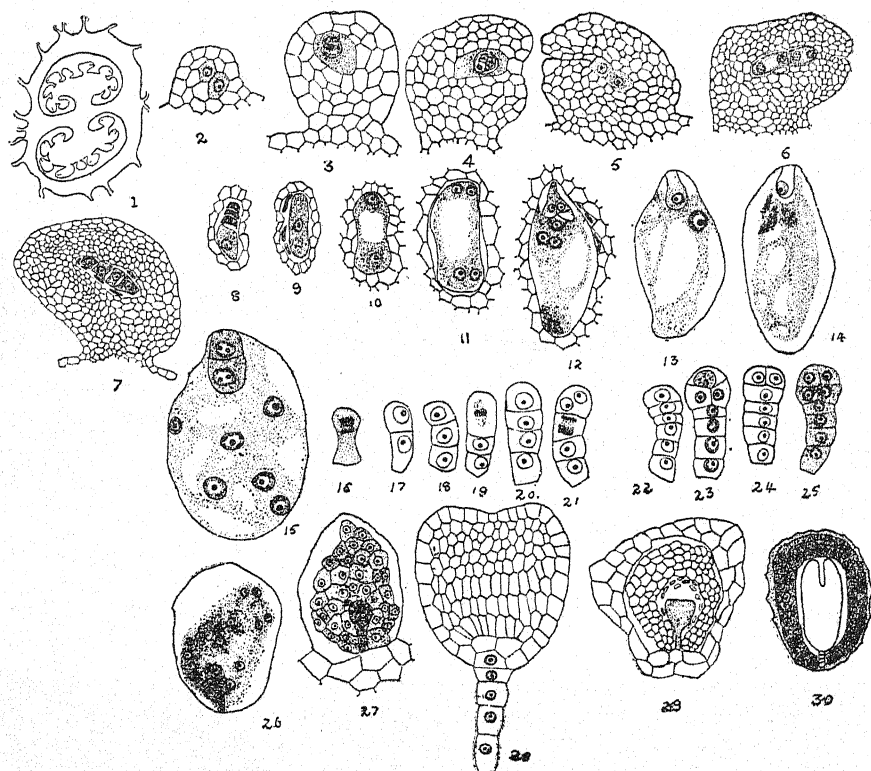
development has been reported in *Scyphiphora* by Karsten (1891). Fagerlind (1937) mentions that *Pepperomia* scheme of embryo-sac development is followed in *Crucianella* and in *Rubia oliverii*.

When the embryo-sac has reached the 8-nucleate stage, 4 nuclei lie at each pole of the embryo-sac. Of these, one nucleus from the chalazal end and one from the micropylar end, travel to the centre and fuse near the egg cell giving rise to the secondary nucleus. The three remaining nuclei at the micropylar end, organize into the egg-apparatus. The two synergidal cells show vacuoles beneath the nuclei, and the egg cell is situated below the synergids at the centre. The three cells at the chalazal end organize themselves into the ephemeral antipodal cells (Fig. 12). Antipodals in the case of this plant have a very short period of existence. Even long before fertilisation they show a degenerating appearance and in many ovules they are not at all found. This fact is very significant because in some other genera antipodals are not only large but through divisions, form large structures and perform haustorial functions (e.g., *Callipeltis*, *Asperula*, *Diodia*, etc.). Fagerlind has classified the type of antipodals according to their size, shape and period of existence, and the antipodals of *Dentella* do not fall into any of these groups described by him. So such ephemeral antipodals if met with in Rubiaceæ may be grouped as the *Dentella* type.

After fertilisation the endosperm nucleus divides first and gives rise to the nuclear endosperm (Figs. 14 and 15). The first division of the zygote is transverse (Fig. 16), resulting in a linear proembryo of two cells (Fig. 17); by another division a three-celled body is formed (Fig. 18). The apical cell divides now giving rise to a four celled proembryo (Fig. 19 and 20). In this four-celled proembryo, both the apical cell and the cell next to that undergo division giving rise to six cells arranged in a linear fashion (Figs. 21 and 22). Now a quadrant is formed through the anticlinal division either of the apical cell (Fig. 24) or of the cell next to that (Fig. 23). The transverse division of each cell of the quadrant results in the octant body (Fig. 25). At this time, the suspensor is uni-seriate and consists of four cells. Longer, uni-seriate suspensors are known in *Richardsonia* (Lloyd, 1902) and in *Spermacoce* (Fagerlind, 1937). Haustorial outgrowths from the cells of the suspensor are characteristic of many genera as examples of which may be mentioned *Asperula*, *Callipeltis*, *Vaillantia*, etc.

The nuclei in the octant cells divide giving rise to two nuclei in each cell (Fig. 26) followed by oblique periclinal wall formation in each cell separating the two daughter nuclei (Fig. 27). The cell of the suspensor immediately attached to the embryo is the hypophysis and it divides periclinally, the daughter cell at the apical portion taking part in the organization of the dermatogen of the embryo. The three layers which will give rise to the dermatogen periblem and plerome are differentiated and the lobing of the cotyledons begins (Fig. 28).

The endosperm formation is nuclear. Wall formation in the endosperm begins only when wall formations take place in the



Figs. 1-30. *Dentella repens* Forst.—Fig. 1. Shows bi-ocular ovary the numerous ovules being midway between amphitropous and orthotropous types. Fig. 2. Two archesporial cells one below the other. Fig. 3. Single archesporium at a later stage. Fig. 4. Megaspore mother cell capped by the single nucellar cell and surrounded by the integument on all other sides. Fig. 5. Dyad stage. Fig. 6. Division of dyad. Fig. 7. Linear tetrad. The micropylar canal cannot be made out. Fig. 8. Shows chalazal megaspore developing and the other three degenerating. Fig. 9. Uninucleate embryo-sac. Figs. 10 and 11. Two- and four-nucleate embryo-sacs. Fig. 12. Mature embryo-sac showing the degenerating antipodals and the polar nuclei about to fuse. Fig. 13. Zygote and the endosperm nucleus. Fig. 14. Division of endosperm nucleus. Fig. 15. Showing the nuclear condition of the endosperm. Fig. 16. Division of zygote. Fig. 17. 2-Cellled embryo. Fig. 18. 3-Cellled embryo. Fig. 19. The same with division of the apical cell. Fig. 20. 4-Cellled embryo. Fig. 21. The same with division completed the cell at the apex and division going on in the cell next to it. Fig. 22. 6-Cellled linear uni-seriate pro-embryo. Fig. 23. Formation of quadrant by the division of the second cell from the apex. Fig. 24. The same by the division of the apical cell. Fig. 25. Octant. Fig. 26. Division of nuclei in the octant cells. Fig. 27. Oblique wall formation in the same. Fig. 28. Embryo showing three suspensor cells and the divided hypophysis. Fig. 29. The same with endosperm cells degenerating around the embryo. Fig. 30. Section of the mature seed showing two or three layers of endosperm cells and a single layer of integumental cells. All the above diagrams are of the magnification $\times 750$, except the following: Figs. 1 and 30, $\times 75$. Figs. 26, 27 and 29, $\times 150$.

octant cells. The wall formation, in the endosperm proceeds centripetally. A lacuna is formed around the embryo through the dissolution of endosperm cells, near the embryo.

In the mature seed the embryo has two cotyledons and lies embedded in two or three layers of endosperm. The integument, even before the lobing of cotyledons, was only two or three layers thick, the rest having degenerated with the growth of the endosperm tissue. In the mature seed it is left as a thin single layer of cells surrounding the endosperm tissue, which stains dark owing to the accumulation of starch and other food substances stored up for the embryo (Fig. 30).

4. *OLDENLANDIA ALATA* Koch.

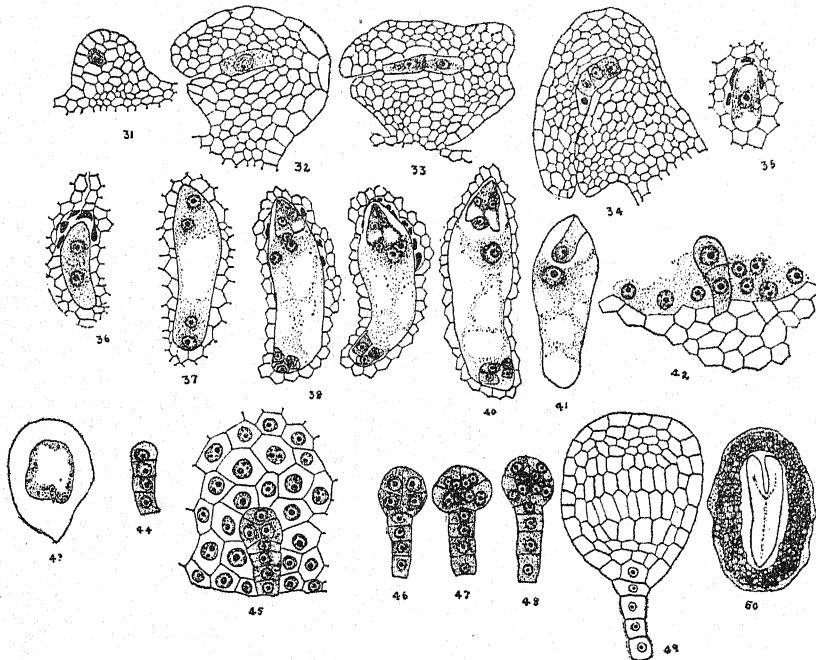
The ovary in this case also is bi-locular with axile placenta bearing many anatropous ovules. The development of the ovule resembles that found in *Dentella*.

A single archesporial cell is differentiated from the hypodermal layer of cells (Fig. 31). In this case also, there is only a single nucellar epidermal cell. This nucellus has been described as belonging to the *Oldenlandia*-type by Fagerlind. The archesporium elongates and undergoes the reduction division giving rise to the dyad (Figs. 32 and 33). The dyad gives rise to the linear tetrad (Fig. 34). By this time, the integument grows fast crushing the single nucellar cell and a very narrow micropylar canal can be made out.

The chalazal megaspore develops into the uninucleate embryo-sac (Fig. 35). The single nucleus divides and the daughter nuclei reach the two poles (Fig. 36). There they undergo two subsequent divisions ultimately resulting in four nuclei at each end. The polar nuclei travel towards the centre and the egg-apparatus is organised (Fig. 38). The polar nuclei fuse near the egg cell (Fig. 39), and the fused secondary nucleus is seen to migrate to a more central position (Fig. 40).

The main feature in which the embryo-sac of *Oldenlandia* differs from that of *Dentella*, is the nature of antipodal cells. Instead of the extremely ephemeral antipodals, the three cells at the chalazal end persist until fertilisation, in good and healthy condition (Figs. 38, 39, and 40), and degenerate only afterwards. But they are not seen to persist for a longer time as in the other genera mentioned before. The embryo-sac is relatively stretched longer and is a bit curved.

The endosperm is nuclear and the first division of the zygote is transverse (Fig. 42). The development of embryo takes place along the same lines as in the case of *Dentella*. In this case, the formation of quadrant was observed to be due to the division of the cell next to the apical cell (Fig. 45) and the division of the apical cell was not observed, while both these methods were found to be followed in *Dentella*. The cell wall formation in endosperm takes place relatively earlier than in the case of *Dentella*. Even in the



Figs. 31-50. *Oldenlandia alata* Koch.—Fig. 31. Single archesporium. Fig. 32. Megaspore mother cell showing the one-celled nucellus, and massive single integument. Fig. 33. Dyad stage. Fig. 34. Linear tetrad. Figs. 35-37. 1, 2, and 4-nucleate embryo-sacs. Fig. 38. Mature embryo-sac before fusion of polar nuclei. Fig. 39. The same showing the fusion of polar nuclei very near the egg cell. Fig. 40. The same showing the secondary nucleus migrated to a more central position. Fig. 41. Zygote and endosperm. Figs. 42-49. Embryo formation: Figs. 42 and 43, showing nuclear endosperm; Fig. 44, cell wall formation in the endosperm. Fig. 50. Mature seed, showing more than four layers of endosperm cells surrounded by a single layer of integumental cells. All the figures except 41 and 50 have been drawn at magnification, $\times 750$; Fig. 41, $\times 500$; Fig. 50, $\times 150$.

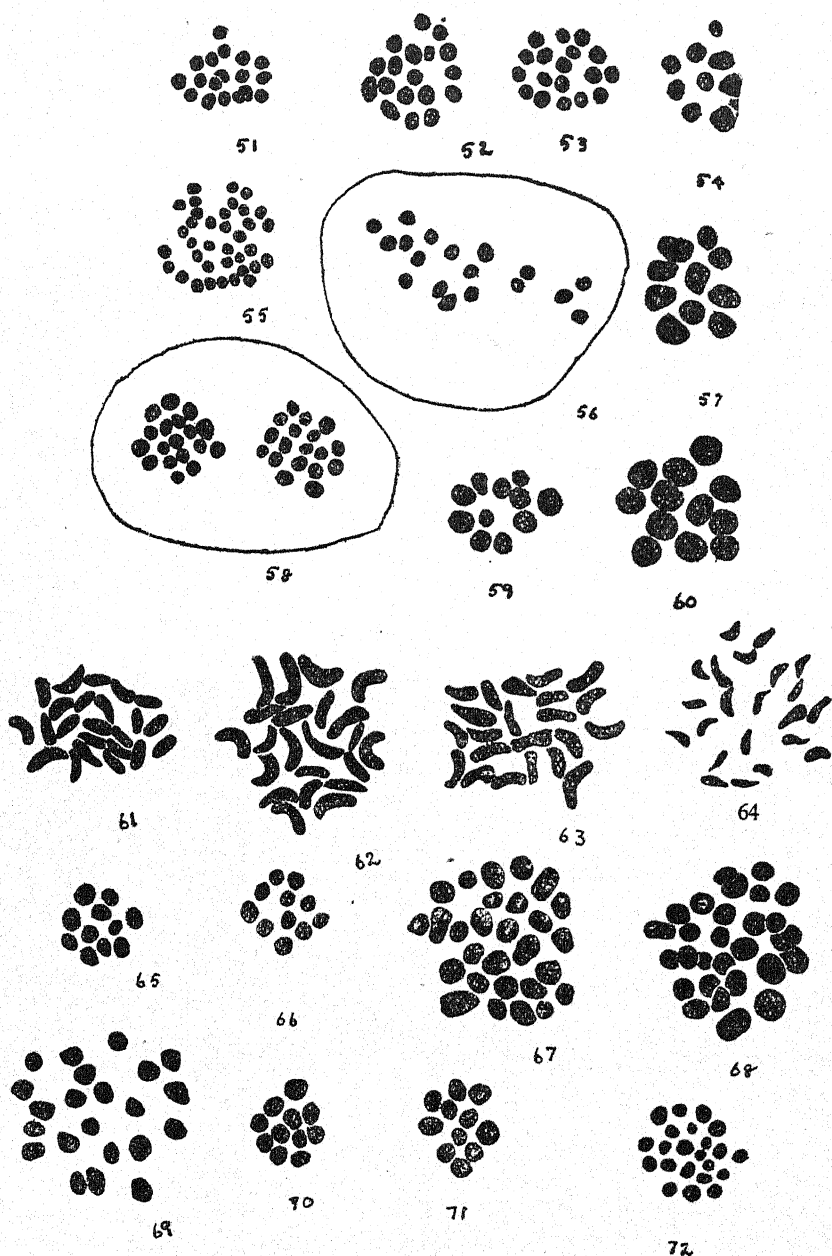
quadrant stage the embryo is seen to be surrounded by endosperm cells (Fig. 45).

As in *Dentella*, the embryo lies embedded in the mass of endosperm tissue around which a thin layer of the integument was observed (Fig. 50).

Thus the genera are very closely related and exhibit extremely similar morphological features, but for some minor differences as the nature of the ovules and the antipodals and the early cell wall formation in endosperm.

5. CHROMOSOME NUMBERS

The chromosome numbers of twenty species distributed over various genera have been determined. It was not possible to determine both the somatic and the meiotic chromosome numbers for all



Figs. 51-72. Chromosome numbers.—Fig. 51. *Oldenlandia alata* Koch. M. I. $n = 18$. Fig. 52. *O. umbellata* Linn., $n = 18$. Fig. 53. *O. corymbosa* Linn., $n = 18$. Fig. 54. *O. crystallina* Roxb., $n = 9$. Fig. 55. *O. paniculata* Linn., $n = 36$. Fig. 56. *O. aspera* D.C., $n = 18$. The chromosomes

the plants. Most of the numbers are determined for the first time and the rest go to confirm the previous findings.

Oldenlandia alata Koch. (Fig. 51) shows the meiotic first metaphase of the plant. Eighteen bivalents are seen and the chromosomes are comparatively small in size.

Oldenlandia umbellata Linn. (Fig. 52) has a haploid number of eighteen as shown in the metaphase I plate.

Oldenlandia corymbosa Linn. (Fig. 53). This plant has also got a haploid set of eighteen bivalents.

Oldenlandia crystallina Roxb. (Fig. 54). This plant shows nine bivalents in first meiotic metaphase.

Oldenlandia paniculata Linn. (Fig. 55). Thirty-six bivalents could be counted in the first meiotic metaphase plate of the plant.

Oldenlandia aspera D.C. (Fig. 56). Shows eighteen bivalents in M. I. plate. The chromosomes are found scattered throughout the spindle region. The exact causes which may be responsible for this could not be found out as the chromosomes were very small.

Pentas carnea Benth. (Fig. 57). The plant during meiosis forms ten bivalents. The somatic chromosome number of this plant has been reported by Fagerlind (1934) as twenty and agrees with the present finding.

Dentella repens Forst. (Fig. 58). Only second metaphase plates were obtained and eighteen chromosomes were counted at each pole.

Ophiorrhiza brunonis, W. and H. Prod. (Fig. 59). The haploid chromosome number was found to be eleven.

Hamelia patens Jacq. (Fig. 60). The somatic chromosome number of this plant was found to be twenty-four by Fagerlind (1937). The meiotic metaphase shows twelve big bivalents and confirms the previous findings.

Mussaenda corymbosa (Fig. 61). The somatic plate shows twenty-two chromosomes which are short. Most of these appear to have terminal constrictions.

Mussaenda luteola (Fig. 66). The meiotic chromosome number was found to be eleven. This is a confirmation of the somatic metaphase count of Fagerlind (1937).

are scattered. Fig. 57. *Pentas carnea* Benth., $n = 10$. Fig. 58. *Dentella repens* Forst., M. II, $n = 18$. Fig. 59. *Ophiorrhiza brunonis* W. and H. Prod., $n = 11$. Fig. 60. *Hamelia patens* Jacq., $n = 12$. Magnification, $\times 4000$. Fig. 61. *Mussaenda corymbosa*, $2n = 22$. Fig. 62. *Gardenia longistella*, $2n = 22$. Fig. 63. *Chomelia asiatica* O.K. ze., $2n = 22$. Fig. 64. *Morinda tinctoria* Roxb., $2n = 22$. Fig. 65. *Morinda tinctoria* Roxb., Meiotic M. I., $n = 11$. Fig. 66. *Mussaenda luteola*, $n = 11$. Fig. 67. *Spermacoce hispida* Linn., $n = 28$. Fig. 68. *S. stricta* Linn., $n = 28$. Fig. 69. *Guetardia speciosa*, $n = 22$. Fig. 70. *Ixora coccinea* Linn., $n = 11$. Fig. 71. *I. finlaysonianana*, $n = 11$. Fig. 72. *Plectronia parviflora* Bedd., $n = 22$. Magnification, $\times 4000$.

Gardenia longistella (Fig. 62). This plant shows somewhat longer chromosomes in the sections of root-tips. There are twenty-two chromosomes.

Chomelia asiatica O. Kze. (= *Webera corymbosa* Willd.) (Fig. 63). This plant also shows twenty-two chromosomes in the somatic plate.

Morinda tinctoria Roxb. (Figs. 64 and 65). The plant shows twenty-two short and slender chromosomes in sections of root-tips. The meiotic metaphase shows eleven bivalents.

Spermocoe hispida Linn. (Fig. 67). Twenty-eight chromosomes were found in the M. I. plate. The chromosomes are very big.

Spermocoe stricta Linn. (Fig. 68). This species also has got a haploid set of twenty-eight big chromosomes.

Guettarda speciosa (Fig. 69). Twenty-two bivalents are formed during meiosis as shown by M. I. plates.

Izora coccinea Linn. (Fig. 70). Fagerlind (1937) found the somatic chromosome number of this plant as twenty-two. This agrees with the number eleven found in first metaphase plates.

Izora finlaysonianana (Fig. 71). Eleven bivalents are seen in the polar view of the first meiotic metaphase.

Platonia parviflora Bedd. (Fig. 72). Twenty-two small bivalents are found in M. I. plates.

6. DISCUSSION

(a) *The nature of nucellus and integument in Rubiaceae.*—The nature of nucellus and integument in the plants belonging to Rubiaceae is not clear. Schleiden (1837) thought that the whole structure of the Rubiaceae ovule is to be considered as a mass of nucellus. But Lloyd is of the same opinion as Warming (1874) that the single thick integument with the very delicate micropylar canal should have misled the earlier observers. According to Houk (1938), the existence of micropylar canal or its absence is not the criterion for judging whether the ovule is naked or not. He thinks that the separation of nucellar cells may result in the formation of a pseudo-micropyle.

The ovule in the two cases described here shows no differentiation of tissues. The mass of cells is homogeneous, and hence the difficulty to distinguish between the nucellus and the integumental portions. Lloyd also has referred to this difficulty in his studies. He says that "the ovules are provided with a single integument and with a greatly reduced nucellus, which is not to be distinguished except at an early stage, when it takes the form of a cap consisting of a single layer of cells crowning the archesporium."

Nucellus in general can be classed under two heads, the crassinucellus and the tenuinucellus. In the case of the crassinucellus, the integuments arise from the basal portion of the body of the

ovules as is found in the polypetalæ. In the case of the tenuinucellus the integument is laid down higher up near the top of the ovule. The tenuinucellus is further classified into two types according to the number of nucellar cells and their nature. The tenuinucellus of the sympetalæ type is of common occurrence in many sympetalous families. It is also found in the genera *Phyllis* (Dahlgren, 1927), *Cephalanthus* and *Chiococca* (Fagerlind, 1937), belonging to the family Rubiaceæ. Here, more than seven to eight cells are seen to surround the archesporium.

In *Oldenlandia*, *Dentella*, and other related genera, the rudimentary type of nucellus or the Orchideæ type occurs. In this case, the ovule is more or less undifferentiated and the ovule consists of only an epidermis or occasionally a few central strings. Even this epidermal nucellar layer is much reduced in the two cases described in this paper. By further reduction, we get an undifferentiated ovule, as is found in *Haustonia*.

From the above said evidence, it is clear that strict differentiation of tissues into nucellus and integuments is not seen in Rubiaceous ovules, and the few epidermal cells capping the archesporium are to be taken as representing the nucellar layer. So there are two possibilities. (1) Houk's interpretation that the integument is suppressed and a pseudomicropyle is formed, (2) Lloyd's view that both the integument and nucellus are present, but are so closely alike or intimately associated, that it is not possible to distinguish one from the other.

The observations recorded in this paper, and the evidence of Fagerlind prove beyond doubt that nucellus is present in a much reduced form along with thick integuments except in the case of *Haustonia*. So Houk's view that the whole body of the ovule represents an undifferentiated nucellus is not in accordance with the above said facts. It may be true in the case of *Haustonia* but in the other cases, Lloyd's view seems to be more in agreement with the facts than Houk's.

(b) *Some cyto-taxonomical considerations.*—The basic chromosome number of a family can be inferred through the comparison of the morphological features of the somatic chromosomes and the identification of ancestrally related chromosome sets which have duplicated themselves, or through the study of the meiotic behaviour of the chromosomes in inter-varietal, inter-specific, and inter-generic crosses. In the latter method, the homology of the chromosomes is more clearly revealed, by the synaptic behaviour and secondary association of chromosomes. Wanscher (1934) from a statistical study of the chromosome numbers of 44 angiospermous families, came to the conclusion that all members had their origin from a four-system. The method of origin of other numbers is through loss or gain of chromosomes, to form, ascending, descending, multiple or secondary polyploid series of chromosome numbers. But Babcock (1934) criticising Wanscher's conclusions, says that a mere statistical analysis of chromosome numbers is inadequate

to determine the basic number of a family. Other features, morphological, embryological and ontogenetical should be taken into consideration, before determining the primitive number of the family, from which the other numbers could be supposed to have arisen.

Examined in the light of chromosome numbers, eleven should be the basic number of the family Rubiaceae, for, most of the Rubiaceous genera exhibit 11 and its multiples as the chromosome numbers of their species. From the data given in this paper, in Tischler's list (1938) and in the list of Fagerlind (1937), it could be gathered that, representative species of fifty-five genera have been investigated. The numbers characteristic of these genera, and the frequency of the genera that exhibit the respective numbers are tabulated below.

Number of chromosomes ..	9	10	11	12	14	17	Total number of genera
Number of genera in each class ..	6	10	33	3	2	1	55

Some of the genera exhibit two numbers, e.g., *Crucianella* shows 9 and 11, and *Galium* shows 10 and 11. But it is clear that 11 is the number found in the maximum number of genera. But this is not a sufficiently strong proof that it is the basic number of the family. For, in *Crepis*, though the haploid number four was found to be widely prevalent, in many species, the number found in the most primitive members of the genus as determined by comparative morphology was found to be $n = 5$. So 5 was considered to be the basic number of the genus (Babcock, 1934). Therefore, further evidence is necessary to infer the basic number of the family.

The nature of the nucellus, or the nucellar epidermis capping the archesporial cells is found to show a phylogenetic significance. Reduction in the number of cells constituting the nucellus, is considered to be an advanced feature. The *Phyllis* type according to Fagerlind (1937) is the most primitive type of nucellus in the family, having a convex layer of seven or eight cells covering the archesporium. The reduction in the number of cells determines the advanced nature of the genera. The *Bouvardia* and the *Oldenlandia* types of nucellus are derived from the *Phyllis* type. In *Bouvardia*, the nucellus is reduced to two to three cells, while in the *Oldenlandia* type it is one celled. *Dentella* also shows only one cell. In the case of *Houstonia*, reduction is carried to the utmost degree so that there is no nucellar epidermis found at all. If this feature is taken as a criterion, the genera *Oldenlandia*, *Bouvardia*, *Dentella* and *Houstonia*, which show nine as the chromosome number, should be considered as phylogenetically more advanced, than other genera showing eleven. According to Lloyd, the genera *Crucianella* and *Vaillantia* also show such nucelli and they have nine

chromosomes, though the former shows eleven in some species. Cases deviating from the above-cited instances occur but they are only very few. *Ophiorrhiza* and *Pentas* show reduced nucelli but have eleven and twelve chromosomes respectively. But the other nine-chromosomed genera show morphologically advanced characters as far as the nucellus is concerned.

Fagerlind (1937) says that the primitive type of nucellus is met with in *Psychotria*, *Cephalanthus* and *Chiococca*. Some other genera as *Canthium* and *Guettarda* also show this feature. All the above said genera show eleven bivalents. So it follows that the primitive type of nucellus is essentially met with in the eleven-chromosomed genera. This gives additional support that eleven might be the more primitive number. From the evidence cited above, it is presumed that eleven is the most probable basic number of Rubiaceæ. But this cannot be said with certainty until and unless the results of more detailed morphological investigations corroborate it. That eleven is a higher number than others occurring in the family, cannot go against its being taken as the basic number. In *Crepis*, though five is a higher number, it is considered to be the basic number in preference to four (Babcock, 1934).

It would thus appear that eleven is the possible basic number of the family. It also seems likely that allopolyploidy has played but a very insignificant part in the origin of species in this family. For this, we have at present only an evidence of a negative kind. If the lowest prevailing number in a family, or some other still lower number possessed by a hypothetical ancestor, is the basic number, and, if allopolyploidy has played a prominent part in the formation of the species, then, one would naturally expect to see secondary association of some kind or other. This phenomenon must especially be evident in a family like Rubiaceæ where the chromosomes are small, and secondary association is not a thing that could easily escape one's attention, for, if it occurs it is unmistakably noticeable as reported in previous cases where this phenomenon has been observed, e.g. *Capparis* (Raghavan and Venkatasubban, 1940), *Crataeva* (Raghavan and Venkatasubban, 1939), *Gynandropsis* (Raghavan, 1938) and *Angelonia* (Raghavan and Srinivasan, 1940). In these, there was hardly a plate that did not manifest secondary pairing. In this family about twenty species have been investigated and though a number of M. I. plates were examined none of them showed secondary pairing. It seems therefore likely, that allopolyploidy has not been the main factor in the evolution of the species belonging to this family. As indicated already, eleven is likely to be the primary basic number and the other numbers including lower numbers like nine might have arisen by various means. Of the various processes involved in producing changes in the germinal material, the following are usually the most important from an evolutionary point of view: (1) Changes in individual genes; (2) changes in sizes and shapes of individual chromosomes, i.e., loss and addition of individual genes or groups of genes through transformation (translocation, inversion, deletion and duplication); (3) changes in groups of

chromosomes through transformation, polyploidy and inter-specific hybridization. Since genic variation consists mainly of internal changes, physical or chemical, in the genes themselves, this might appear to be the basic process of evolution. On the other hand, genic variation may never induce chromosomal variations. For example, in *Crepis* (Babcock and Navaschin, 1930) it was proved that the diversity came about only through the transformational processes which induced changes in chromosome number and morphology. From this point of view transformation is the basic evolutionary process. In some of the minor groups of related species, however, a diversity has come about through changes affecting whole groups of chromosomes. Following these hypothetical major steps, and within the new forms so derived, both genic variation and chromosomal translocations would operate to induce differentiation of new species with different chromosome complexes. From this point of view polyploidy and interspecific hybridization would represent the more basic processes. In this family there is no doubt that this last named process is responsible for the origin of some species with 9, 18 and 36 chromosomes. But this fails to account for the 9, 10, 12 portion of the series found in this family. Since genic variations cannot account for this it is suggested that transformational processes have been responsible for these chromosome numbers and the absence of any secondary pairing makes one infer, at least tentatively, that in the polyploid series exhibited by several species in the family, autopolyploidy rather than allopolyploidy has been the main factor.

7. SUMMARY

The embryo-sac formation and embryo development have been described in *Dentella repens* and *Oldenlandia alata*.

The chromosome numbers of twenty species have been reported most of which have been determined for the first time.

The nature of nucellus and integument is discussed in the light of observations recorded in this paper.

The probable basic number of this family is discussed on the basis of morphological features and the methods by which the various numbers might have originated are discussed.

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MORPHOLOGICAL STUDIES IN ORCHIDACEÆ

I. *Zeuxine sulcata* Lindley

BY K. N. SESHAGIRIAH, M.Sc.

Department of Botany, Central College, Bangalore

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DURING a morphological study of this plant it was noticed that all the flowers in an inflorescence set seeds though the pollinia of the flowers were not removed from them. It was thought that the pollinia from the other plants must have been carried by insects and cross-pollination must have been effected. But closer observations revealed that there were no insects bringing about pollination and in all the plants in the locality the pollinia were not detached from the flowers. This clearly proved that pollination did not occur in these plants. Microscopic examination of the pollinia from the flowers just opened showed in some cases normal pollen grains and in others degenerated ones. Germination experiments of the pollen proved negative.

Pollinia from the older flowers in which the pistil had already set seeds showed an abundant development of an intra-cellular fungus. These features were interesting specially in view of the fact that in the Orchidaceæ the stimulus of pollination is known to be necessary not only for the initiation of the ovules in some cases but also for the further development of the ovules. Hence a detailed morphological study was undertaken and two short notes were published by the author (1932 and 1934). Later more material was prepared to verify the previous work and the result is embodied in the following pages.

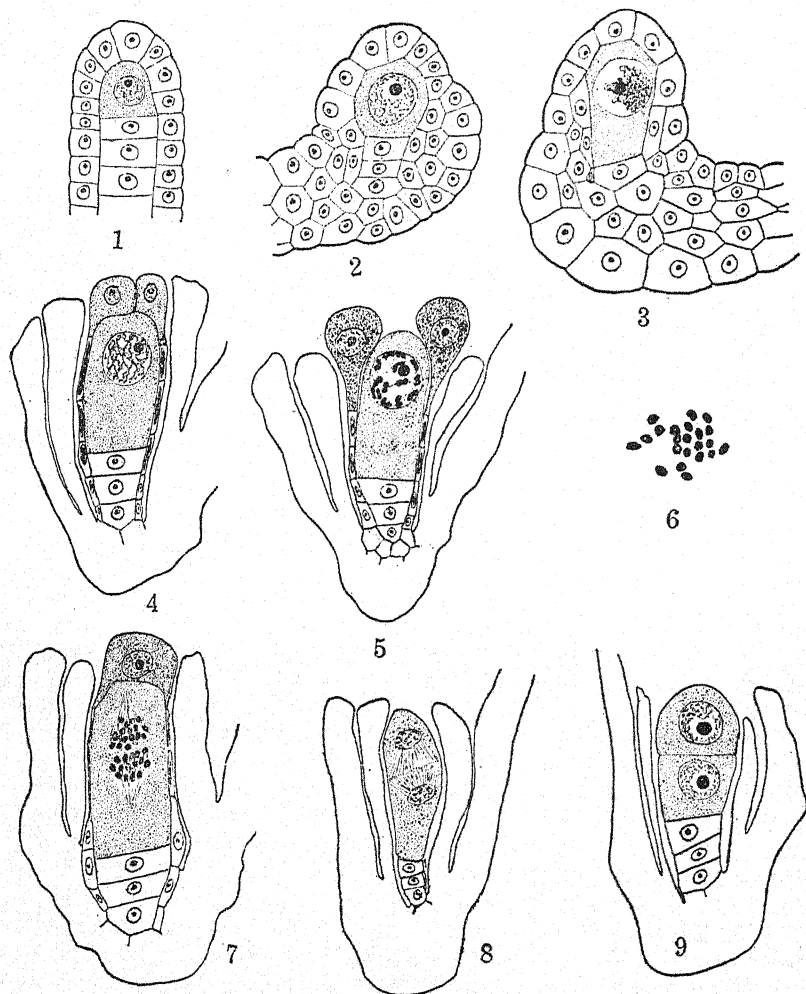
Regarding the previous work on Orchidaceæ, Schürhoff (1926) and Schnarf (1929) have given a general review of the work done from a very early time. Strasburger, Treub and Guignard (1879-1889) were the earliest contributors to the studies on this family. Later on, Baranow, Afzelius, Vermoessen, Magnus, Kusano, Sharp, Brown and Pace (1900-1929) have added more to our knowledge. So far no work has been done on the genus *Zeuxine*.

Material for this investigation was collected near Bangalore during the months of September and October and fixed in Flemming's fixative and the usual xylol-paraffin technique was followed. Sections were cut from 8 to 12 μ in thickness and were stained in iron-alum hæmatoxylin.

OBSERVATIONS

The archesporial cell (Fig. 1) which functions directly as the megaspore mother cell is initiated and the ovule begins to develop long before the flower opens. The prophase changes that occur in the nucleus of the megaspore mother cell appear to be normal. There is a distinct synizetic contraction (Fig. 3) followed by the

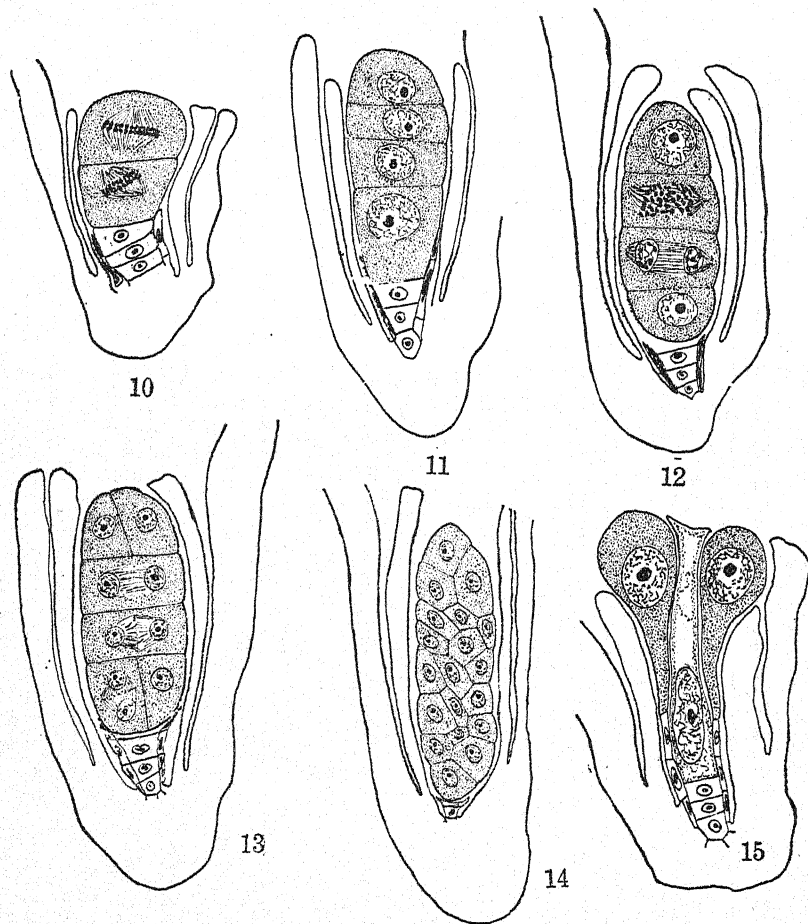
open spireme stages (Fig. 4) and bivalent formation of chromosomes, which lie well distributed in the nuclear area during diakinesis



Figs. 1-9.—Fig. 1. Nucellar primordium showing the archesporial cell. $\times 600$. Fig. 2. Young ovule, showing the developing megaspore mother cell. $\times 600$. Fig. 3. Nucleus of the megaspore mother cell in the synizetic contraction. $\times 600$. Fig. 4. Mother cell. Nucleus in the open spireme stage. ($\times 600$). Fig. 5. Mother cell. Nucleus in the diakinesis stage. The distal nucellar cells have disintegrated, the laterally situated nucellar cells are enlarging. $\times 600$. Fig. 6. Transverse section of the heterotypic metaphase plate showing the 22 bivalent chromosomes. $\times 600$. Fig. 7. Heterotypic anaphase. The two distally situated nucellar cells have fused with each other prior to their degeneration. $\times 600$. Fig. 8. Heterotypic telophase. Nucellar cells have degenerated. $\times 600$. Fig. 9. Two daughter cells. Nucellar cells have completely degenerated and hence the nakedness of the cells. $\times 600$.

(Fig. 5). A transverse section of the metaphase plate (Fig. 6) shows 22 bivalent chromosomes. During the anaphase (Fig. 7) the chromosomes are not always pulled apart simultaneously; but ultimately reach the two poles (Fig. 8) and two distinct daughter cells are formed (Fig. 9).

The daughter cells after a period of rest divide homotypically (Fig. 10). The row of cells, that results after the homoeotypic division, corresponds to a linear tetrad of megaspores (Fig. 11). These cells divide further (Figs. 12 and 13) and directly form an embryo (Fig. 14) without going through the usual processes of the degeneration of some of the megaspores, the formation of the embryo-sac and fertilization.

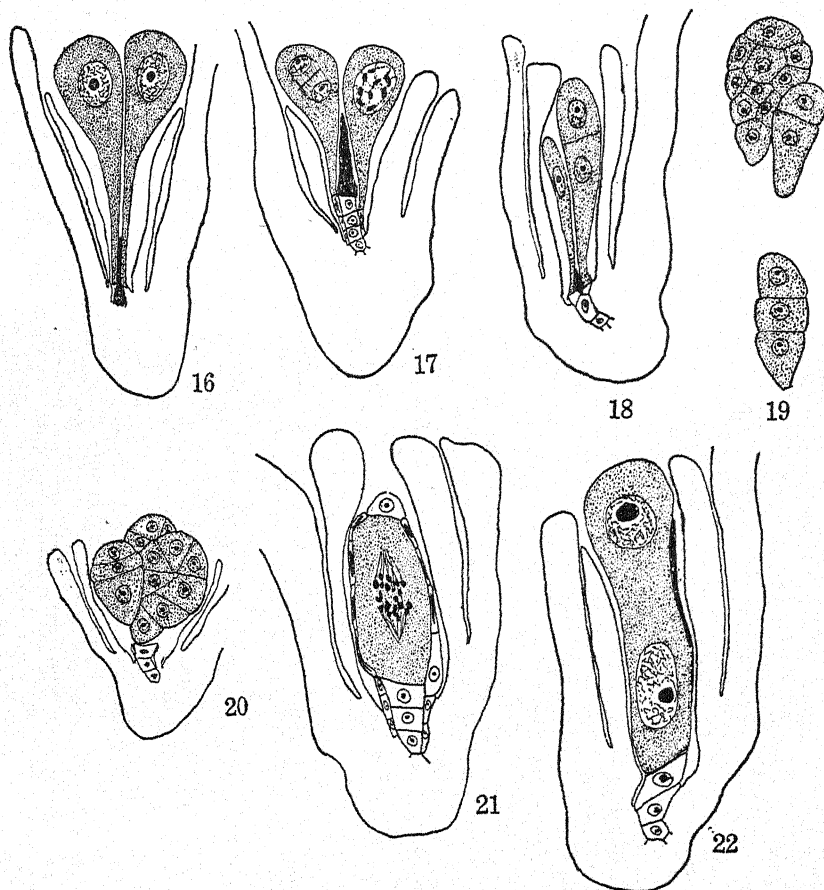


Figs. 10-16.—Fig. 10. The two daughter cells dividing. $\times 600$. Fig. 11. The linear tetrad of megaspores. $\times 600$. Figs. 12 and 13. The megaspore cells dividing. $\times 600$. Fig. 14. An adult embryo $\times 600$. Figs. 15 and 16. The nucellar cell enlarging. In Fig. 15 the megaspore mother cell, which has enlarged much, is decaying. $\times 600$.

In a few cases, it was seen that, the mother cell goes through the earlier stages (Figs. 21 to 24) in the formation of the embryo-sac, where the sac develops from the mother cell itself. In one case it was also observed that after the formation of the linear row of four cells, corresponding to the tetrad of megaspores, the lowermost cell (Fig. 25) among them exhibits somewhat a general organisation of an embryo-sac with a number of free nuclei evidently derived by the division of the original nucleus of the cell and a large central vacuole. Such ovules probably degenerate.

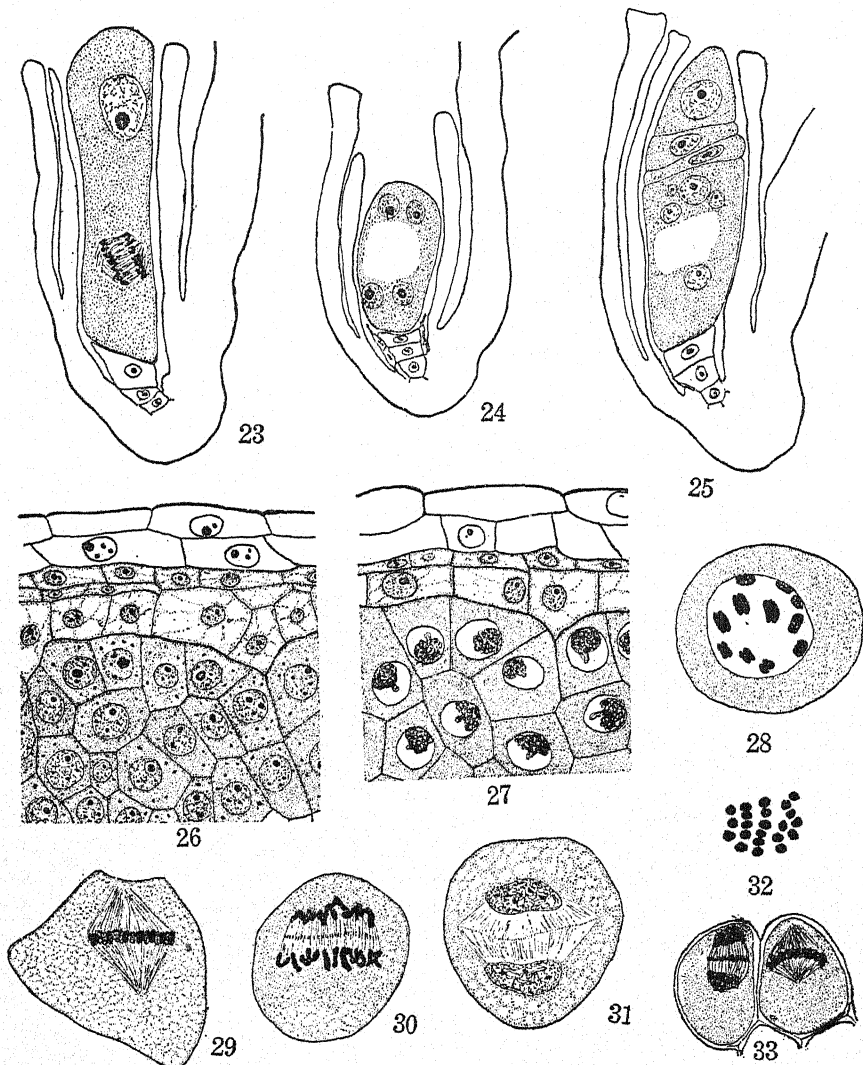
NUCELLAR EMBRYONY

Embryos develop from the nucellar cells (Figs. 15 to 18) situated on the sides of the mother cell. These cells increase in size and divide (Fig. 17). Very often the megaspore mother cell decays



Figs. 17-22.—Figs. 17 and 18. Stages showing the division of the nucellar cells to form the nucellar embryos. $\times 600$. Figs. 19 and 20. Groups of three embryos in the ovules. $\times 600$. In Fig. 19 the integuments of the ovule are not shown. Fig. 21. The megaspore mother cell. Nucleus in the heterotypic anaphase. $\times 600$. Fig. 22. Two-nucleate embryo-sac. $\times 600$.

and the embryos develop from the nucellar cells only (Figs. 15 to 18). In a fully developed seed, two to three embryos can be easily made out (Figs. 19 and 20). Embryos developed from the nucellar cells are presumably diploid. Afzelius (1928) has reported similar



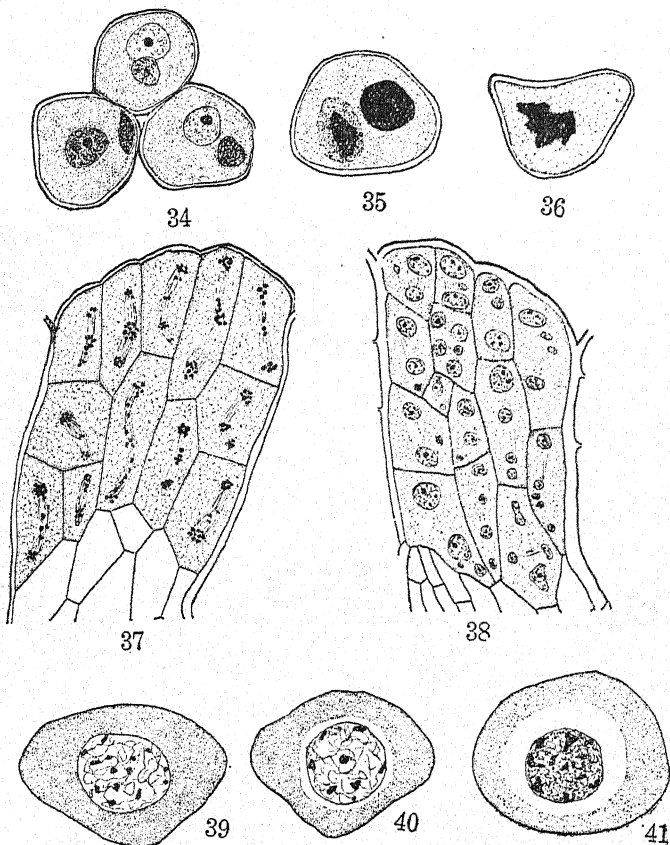
Figs. 23-33.—Fig. 23. Division of the lower nucleus in the two-nucleate sac. $\times 600$. Fig. 24. Four-nucleate embryo-sac. $\times 600$. Fig. 25. A row of four megaspores in which the lowermost megaspore shows the general organisation of an embryo-sac. $\times 600$. Figs. 26 and 27. Part of the pollenium showing the microspore mother cells. $\times 420$. Figs. 28 to 31. Various stages of the heterotypic nuclear division. $\times 1200$. Fig. 32. Transverse section of the heterotypic metaphase plate in the microspore mother cell, showing the 22 bivalent chromosomes. $\times 1200$. Fig. 33. "Dyad" of microspores. $\times 900$.

embryo formation from the nucellar cells in *Nigritella nigra*, a member of the Orchidaceæ.

Germination experiments of seeds in *Zeuxine* were not successful in spite of providing the seeds with the necessary symbiotic fungus which was isolated, by means of pure culture, from the roots of adult plants of *Zeuxine*. The only method of propagation appears to be vegetative as reported by Joshi (1933).

MICROSPORE FORMATION

During microspore formation also several abnormal features are seen. In some of the microspore mother cells there is a normal meiotic division (Figs. 26 to 32) while in others belonging to the same pollinium signs of necrosis are seen from an early stage. In such cells (Figs. 39 to 41) the nuclei show a dense reticulum of thin chromatin threads and the cytoplasm gradually withdraws away from the nuclear membrane before the final break down of the cells.



Figs. 34-41.—Figs. 34 to 36. Stages showing the decay of the microspores. $\times 900$. Figs. 37-38. Microspore mother cells showing the abnormal spindles during the heterotypic nuclear division and the formation of supernumerary nuclei. $\times 600$. Figs. 39-41. Stages showing the decay of the microspore mother cells. $\times 1200$.

In the cells that divide normally, 22 bivalent chromosomes (Fig. 32) are seen in the heterotypic metaphase plate. The same number of bivalents were counted during the heterotypic division of the nucleus of the megaspore mother cell also (see page 359).

The second meiotic division seems to be suppressed; hence "dyads" of microspores are formed. These on dividing (Figs. 33 to 36) give rise to a vegetative nucleus and a generative nucleus, but such spores are not functional as they soon degenerate.

In some pollinia abnormal spindles (Fig. 37) are formed during the heterotypic nuclear division, resulting in the formation of non-functional supernumerary nuclei (Fig. 38). Finally the pollinium is attacked by a fungus. The meiotic irregularities in the microspore mother cells and the final pollen sterility are in correlation with the apomictic development of embryos.

DISCUSSION

In apomixis the chief cause is the failure of either meiosis or fertilisation. Sometimes both these processes may fail. Meiotic processes are suppressed in many ways in the megaspore mother cells of apomictic plants, the final result being the unreduced nucleus in the egg, which may develop parthenogenetically, giving rise to the diploid sporophyte.

Cases of haploid sporophytes developed apomictically are also on record where the meiosis in the mother cell and the development of female gametophyte are normal. But haploid sporophytes developed apomictically direct from a tetrad of haploid megaspores has not been recorded so far. According to Chiarugi (1926) in *Artemisia nitida*, which reproduces apomictically, the tetrad of haploid megaspores formed after the normal meiotic process fails to survive. In *Zeuxine sulcata*, the tetrad of haploid megaspores not only survives but continues to divide actively and forms an embryo. Apomictic development of the embryo has been recorded also in *Gastrodia* by Kusano (1915) among the Orchidaceæ. But in *Gastrodia* the haploid sporophyte develops from the haploid egg cell and not direct from a tetrad of haploid megaspores as seen in *Zeuxine*.

Various theories have been advanced to explain the causes of apomictic development in plants. According to Ernst (1918) hybridization is one of the primary causes of apomixis including parthenogenesis, apogamy, apospory, nucellar embryony and polyembryony. The meiotic aberrations that are observed in hybrids are also seen very commonly in apomictic plants. Though hybridisation brings about synaptic and other meiotic aberrations leading ultimately to the sterility of the sexual cells and the apomictic development, it is not the only primary factor which causes apomixis.

Ernst and others (1910) who believe in hybridization theory, do not at the same time deny the importance of nutritive disturbances set up by natural or other causes and its relation to apomixis.

Zeuxine, during its vegetative phase, is partly autotrophic. Its leaves are rich green in colour. They are efficient as photosynthetic organs. In its root system the symbiotic mycorrhizal fungus is seen thriving, but not yet undergoing disintegration.

But later on, as the plant approaches the reproductive phase, it becomes markedly holosaprophytic. Its leaves gradually lose their green colour, turn yellow and their efficiency as organs of photosynthesis is lost. In the root system there is a marked disintegration and digestion of the mycorrhizal fungus, the digested product being absorbed by the plant for its nutrition.

Burgeff's researches (1931) on this aspect of nutrition among the saprophytic terrestrial orchids are in complete agreement with the author's observations. In fact Burgeff includes *Zeuxine* among the orchids that are holosaprophytic during their reproductive phase. It seems that this nutritive disturbance, just prior to the reproductory phase, may set up causes which bring about the phenomenon seen in *Zeuxine*.

Haberlandt (1921a and 1922) advanced his theory of "Nekro-hormones" as the possible cause of apomixis. According to him, wounding of the tissue of the nucellus results in the formation of "Nekrohormones" and these "Nekrohormones" bring about the formation of adventitious embryos in *Oenothera*. So he explained the occurrence of natural apomixis as caused by a similar production of "Nekrohormones" in the degenerating tissue of the embryo sac and the nucellar cells. This explanation seems to be applicable in the case of *Zeuxine* also, where we see in the ovules apomictic development and nucellar embryony along with much nekrosis of the nucellar cells.

SUMMARY

1. The life-history of *Zeuxine sulcata* Lindley, is highly abnormal.
2. The megaspore mother cell after the reduction division gives rise to a linear tetrad of four megaspores which divide further and form an embryo apomictically. There is no degeneration of megaspores and the embryo-sac formation is entirely omitted. The embryo thus developed is haploid.
3. There is nucellar polyembryony. The embryos developed from the nucellar cells are diploid.
4. During microspore formation several abnormal phenomena occur, as a result of which there is pollen sterility.
5. The haploid number of chromosomes is 22.
6. It is shown that *Zeuxine* is autotrophic during the vegetative phase and holosaprophytic during the reproductive phase. The apomictic and the other abnormal phenomena seen in this plant are explained as possibly due, either to the nutritive disturbances which occur prior to the reproductive phase or to the effect of "Nekro-hormones" from the nekrotic cells that are seen in the ovules.

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